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WHAT ARE NEUROFIBRILS?[1]

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NERVE cells and reproductive cells share honors in being the most complex cytological elements in the bodies of the higher animals. The cellular nature of the egg and of the spermatozoon was early recognized by the pioneer workers in the field of animal cytology, but it required over half a century of investigation to make clear the true character of the nervous elements. The cell theory, proposed by Schleiden and Schwann in 1838–39, was not successfully applied to nervous tissues till 1891. Before that time nerve fibers, ganglion cells, fibrillar material, sense cells, and the like afforded a confused mass of elements which were assumed to serve as a basis for nervous organization.

Nerve fibers were apparently first described by Fontana in 1781. Ganglion cells were first identified in 1833 by Ehrenberg, who published a more elaborate account of these bodies in 1836. Five years after the discovery of these cells, that is in 1838, Remak declared that nerve fibers could be traced directly from ganglion cells, and this conclusion was supported by Helmholtz (1842) and by Hannover (1843). In the work of all these investigators, however, it was not absolutely certain that the fibers found by them in connection with the ganglion cells were really nerve fibers. This uncertainty was removed by the discovery of Koelliker (1844) that in the vertebrates certain medullated fibers, about whose nervous

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nature there was not the least doubt, could be shown to be in direct continuity with ganglion cells.

In 1855 Leydig pointed out in his study of the central nervous organs in spiders that these organs contain in addition to nerve fibers and ganglion cells masses of finely fibrillar material to which he gave the general name of punctate substance. Material of this kind was subsequently recognized as a constant ingredient in the gray portions of the spinal cord and of the brain in the higher animals. The interrelations of the punctate substance and particularly of the nerve fibers and the ganglion cells occupied the attention of students in this field of investigation from the middle of the nineteenth century till toward its end. In 1891 Waldeyer announced the neurone theory, a theory that united in a truly remarkable way a vast array of facts brought to light by the earlier investigators.

According to this theory the nervous system is composed of numerous anatomically and genetically interrelated units or neurones, each one of which consists ordinarily of three sets of parts: a nerve-cell body, one or more nerve-fiber processes and numerous finely divided dendritic processes. Such a cell body with its processes constitutes a true nerve cell or neurone. The year 1891 may therefore be looked upon as that in which the cellular interpretation of the nervous elements was first successfully accomplished, for the doctrine of the neurone has been to all intents and purposes universally accepted by the most competent students in the field of general neurology.

While the discoveries that led to the neurone theory were being made, the finer structure of the nervous elements was also being investigated. By such older workers in this field as Treviranus (1816), Ehrenberg (1833, 1836) and Valentin (1836), the contents of nerve fibers and of ganglion cells was believed to be a finely granular material of almost fluid consistency. In 1843 Remak called attention to the fact that in addition to this granu-

lar material the larger nerve fibers in the nerve cord of the crayfish possessed an axial bundle of some hundreds of very fine fibrils which ran in parallel courses along the length of the fiber. According to Remak these fibrils were best seen in the fresh condition of the fiber. Soon after a preparation of them had been made they disintegrated and the resulting granular material showed no trace of them.

In several papers published in the decade following 1863 Max Schultze enlarged upon the nature of the fibrils discovered by Remak. He believed that these bodies could be identified with certainty not only in the axis cylinders of nerve fibers but also in the substance of ganglion cells. Writing of the large cells in the brain of the Torpedo he stated (1871) that "it was most convincingly shown here that the large cells removed from the living animal, and prepared in serum, in which they were capable of being easily isolated, possess, both in their processes and in their proper substance, an exquisitely delicate fibrillar structure." He further maintained "on physiological grounds . . . the possibility of isolated conduction in these constituent fibrils." It is obvious from these quotations that Schultze believed not only in the presence of neurofibrils in ganglion cells and nerve fibers but also that these fibrils are in fact the structures immediately concerned with that activity which is most fundamental of all nervous operations, namely, conduction. From this standpoint Schultze may be looked upon as the founder of the neurofibril hypothesis.

Yet, notwithstanding Schultze's clear statement of this hypothesis, his ideas attracted very little attention. To the most expert of the early workers neurofibrils were at best only just within the range of visibility, and their identification and investigation were consequently matters of extreme difficulty. Often sought for, they were seldom found and consequently an attitude of suspicion grew up about them that led many to deny their existence altogether. This disbelief in the reality of neurofibrils

was a common opinion at that time, notwithstanding the fact that a reasonably satisfactory staining method for their demonstration had been devised by Frommann (1864) and later a much more satisfactory one by Kupffer (1883). But most of the new methods for the study of nervous tissues, as for instance the Golgi silver impregnation method (1875) and the methylene-blue technique (Ehrlich, 1886), were directed toward the grosser relations of fibers and cells rather than toward the finer structures within these parts. The period from the early seventies when Schultze made his mature declarations about neurofibrils till the early nineties was a sterile one so far as neurofibrils were concerned. This period culminated in the nineties in what Bethe appropriately called the "Golgi Enthusiasmus."

But the neurofibril hypothesis even at this time was not without its earnest advocates. In 1889 Apáthy issued a preliminary paper on the direction in which neurology should be reformed, and in 1897 he published the first and what proved to be the only part of an extended monograph on the transmitting elements of the nervous system and their relation to the cells. In all of Apáthy's discussion the neurofibril is the fundamental structure. By several new methods of staining, particularly one based on the use of gold chloride, he succeeded in demonstrating neurofibrils with a clarity and distinctness such as had never been approached before. With the clearness of metallic wires neurofibrils could be traced in his preparations through tissue of unusual transparency. Unfortunately the methods by which these preparations had been made were extremely precarious. In the hands of most workers they yielded almost nothing, and even Apáthy himself confessed that his own best efforts commonly resulted in complete failure. Nevertheless when the preparations were successful they exhibited neurofibrils with remarkable clearness.

In such preparations, which were based in the main on material from the leech, the earthworm and like animals,

neurofibrils could be traced, according to Apáthy, through sense cells, around whose nucleus they commonly formed a network, over nerves and into the central nervous organs. Here they branched profusely, forming true networks, and as delicate fibrils they entered the bodies of unipolar ganglion cells to form a complicated fibrillar network extending through most of the peripheral protoplasm. From this network branches made their way inward toward the nucleus of the ganglion cell around which was formed a second or inner net, and from this inner net a coarse neurofibril emerged from the ganglion cell, and, after having passed through the central nervous organ, again made its way over a given nerve to a muscle which was thus innervated. In this way continuous neurofibrillar material was believed to extend from a sense cell on the surface of the animal through the central organ to a termination in a distant muscle. Thus the neurofibril rather than the cell was in Apáthy's opinion the all-important element in the organization of the nervous system.

Apáthy's conception of nervous organization thus laid stress on a continuous neurofibrillar material and relegated nerve cells to a very subordinate position. In both these respects it was in radical opposition to the neurone theory. According to this theory nerve cells are the real units of nervous organization. In their early developmental stages as neuroblasts they had been shown to be distinct and separate bodies whose subsequent union was established through the outgrowth of processes which eventually reached from cell to cell. It was the opinion of most neuronists that the processes from one cell never really fuse with those from another but that they are at most simply in contact, a contact which in fact from time to time may even be broken and then reestablished. Such points of contact, the so-called synapses, were definitely shown to be polarized, in that nerve impulses can pass over them in one direction but not in the other. Thus the advocates of the neurone theory emphasized a degree of

discontinuity between the nerve cells that was in strong opposition to what Apáthy maintained.

As a result of these differences of opinion a lively debate arose on the question of neural continuity or discontinuity. While this discussion was in progress two new and important methods for the demonstration of nerve cells and neurofibrils were devised. These were Bielschowsky's ammonia-silver method first announced in 1902 and Ramón y Cajal's reduced silver method described one year later. Both methods were modified in a variety of ways and both were so successful as means of demonstrating neurofibrils that these bodies became the objects of active inquiry in many neurological laboratories. The problem that excited the interest of most workers was that already indicated, the degree of continuity between the nervous units.

It must be admitted, however, that the discussions that took place as a result of these differences of opinion led to no very definite conclusions. Many workers had the opportunity of examining at first hand the remarkable preparations made by Apáthy, but few were able to convince themselves that the preparations gave evidence of the kind of continuity claimed by him. Preparations that had been made in Bielschowsky's laboratory and that seemed entirely convincing to this worker failed to meet Ramón y Cajal's requirements of what a demonstration of continuity should be. Thus in general great uncertainty prevailed.

As a possible solution of some of these difficulties it was suggested that the nervous systems of different animals might be differently organized. In the higher forms where the synaptic system prevails discontinuity might be the rule. In the lower forms where the nerve-net is common continuity might obtain. Such an opinion was expressed by Sherrington (1906) and was subsequently elaborated by Parker (1919). To what extent this view is sound is perhaps open to question, for Bozler (1927) has recently given good reason for believing that the cel-

lular elements in the nerve-net of the jellyfish Rhizostoma are quite as disconnected as is assumed for the synaptic system. Should this discovery by Bozler prove to be well founded, it may result in the complete overthrow of the idea of continuity, for, if the synaptic nervous system is composed of discrete units and the same is shown to be true of nerve-nets, continuity as represented by continuous neurofibrillar substance between nerve cells would cease to be tenable. The synaptic system would differ then from the nerve-net only in that the former is polarized in the direction of its conduction and the latter not. In such a case neurofibrils in the nerve-net as well as in the synaptic system would be strictly intracellular structures.

As a result of the introduction of the methods of Bielschowsky and of Ramón y Cajal great additions were made to what had already been known concerning neurofibrils. They were identified in the nerve-cells of a wide range of animals, in receptor organs and in the motor apparatus. Their pathology was extensively investigated and perhaps most important of all their developmental history was worked out. From these studies three important conclusions may be drawn: first, neurofibrils develop at a stage much in advance of functional activity either sensory or motor; second, they begin their growth close to the nucleus of the neurone (Cowdry, 1914); and, finally, they do not grow beyond the limits of a neurone, that is, they are strictly intracellular (Gerini, 1908).

Most intimately associated with the modern investigation of neurofibrils is the work of Bethe which, beginning about three decades ago, extended to within the last ten years or so. By novel methods, including the use of such delicate stains as toluidin blue, this indefatigable worker confirmed many of the details of Apáthy's investigations. According to Bethe (1900) neurofibrils maintain in general independent courses over nerve fibers or through nerve cells, but in certain places true nerve-nets may be present. In agreement with Apáthy, Bethe declared for

the continuity of neurofibrillar material, and his views in this respect were criticized in much the same way as those of Apáthy had been. Bethe further vigorously advocated Schultze's original declaration that neurofibrils are the transmitting elements in the nervous system.

To this end Bethe offered two interesting pieces of evidence. In the first of these he followed a line of experimentation devised somewhat earlier by Jenkins and Carlson (1904). In these tests he attempted to determine the length of time required for a nerve impulse to pass over a given extent of nerve first in a relaxed state and then in a slightly stretched one. The preparation used was the ventral nerve chain of the leech, and Bethe claimed that the time of transmission remained the same for the lax and for the stretched state. Since the neurofibrils are tortuous in the lax condition and approximately straight in the stretched one it follows that the evidence favored the opinion that conduction is accomplished by these bodies rather than by the neuroplasm or other relatively fluid parts of the fiber which do actually change in length with relaxation and with stretching. Bethe's results, however, were in opposition to those of Jenkins and Carlson (1904) and of Carlson (1905) alone in all of which work it was shown that the time of transmission did increase with stretching, a condition which indicated that the transmitting agent was fluid rather than solid. In consequence of this fact as well as of the further one that Bethe's observations were carried out on a piece of central nervous organ and not on a bundle of nerve fibers as those of Jenkins and Carlson were, Bethe's experimental test has not been accepted as crucial.

A second line of evidence introduced by Bethe (1910) turned on the condition presented by the neurofibrils at certain points in the course of medullated nerve fibers. Bethe believed in common with Mann (1898) that at the nodes of Ranvier in these fibers the fluid neuroplasma was completely interrupted and that the only elements that passed through this region were the neurofibrils. Such a state of affairs would of course point to the fibrils as the transmitting parts. But the work of such histolo-

gists as Boveri (1886) on the structure of the nodes of Ranvier did not support the opinion of Bethe nor did this opinion seem tenable to more recent students of the subject. Hence Bethe's second effort to prove that neurofibrils are the conductors of the nervous system failed to be convincing.

In a cursory survey of the work on neurofibrils covering the ninety years that have elapsed since their discovery it must be evident that students of these structures have come to rely more and more on complicated and intricate methods for the demonstration of these bodies. The discoverer Remak claimed that he saw these fibrils best in fresh cells and fibers. Schultze, their chief advocate, described them from fresh tissue or from that examined in serum. Then came the attempts of Frommann and of Kupffer to stain the fibrils specifically, followed by the brilliant but precarious method of Apáthy, which gave way to the rather intricate but more reliable means of demonstration devised by Bielschowsky, Ramón y Cajal and Bethe. But any one who has worked even with these more recent methods must have been impressed with their precariousness. Tello (1922), using the Rámon y Cajal procedure, was able to demonstrate a fibrillar system in connective tissue cells as well as in nerve cells. All workers were familiar with the fact that different preparations made by the same method commonly showed great differences in the details of the fibrils and even in the same preparation the fibrils of different parts differ greatly among themselves. This diversity of appearance has been made the subject of critical comment by Clivio (1927), and is the chief basis for that skeptical attitude that many modern workers maintain toward the whole problem. Thus Bayliss (1914) in his "Principles of General Physiology" expressed the opinion that the so-called neurofibrils are pure artifacts produced by the methods employed in the histological preparation of the nervous tissue in which they are seen and that they are in no sense constituents of the living cell. This opinion had also

been entertained by Pighini (1908). W. H. Lewis and M. R. Lewis (1924), both experienced cytologists, reached much the same conclusion. Cowdry (1928), on the other hand, after admitting the great uncertainty that is to be attributed to the methods by which neurofibrils have been demonstrated remarks that these bodies must have some material basis in the cell and that they can not be due altogether to technique. Somewhat the same opinion as Cowdry's was expressed by Bocke (1926), who admitted the existence of neurofibrils in living tissue but in forms other than those seen in preparations.

A solution for these difficulties is not easily found. The fact that neurofibrils can be demonstrated by a great variety of methods is not conclusive proof, as some have thought, that these bodies are constituents of living cells, for all such methods involve coagulation. Nor does the demonstration of neurofibrils in tissue instantly killed in hot water (Lugaro, 1909) show that the fibrils were present before the killing. In fact no evidence is conclusive on this point except that which can be drawn from living material. What can be demonstrated in the living cell is surely there. Much of the evidence from living cells dates from the time of Remak and Schultze but within the last two years a very important addition has been made to this body of fact by Bozler (1927).

This investigator has shown that in the large living nerve cells in the circular nerve band of the jellyfish Rhizostoma it is possible to demonstrate with remarkable clearness a system of neurofibrils. These fibrils run lengthwise in the bipolar cells that make up the subepithelial nervous tissue in this jellyfish. When this animal is placed in slightly hypertonic sea-water, its nerve cells in consequence of the general shrinkage of its tissues shorten and thus throw the bundle of contained neurofibrils into folds. Under such circumstances the neurofibrils, often partly isolated, can be seen with great clearness in the cells, and yet in this state the tissue is still living for the jellyfish as a whole continues to pulse and

on being returned to ordinary sea-water it makes a complete recovery.

Another remarkable fact noted by Bozler is that after the Rhizostoma has been for some time in hypertonic sea-water small hernia-like enlargements appear on the surfaces of its nerve cells. These sacs are formed in consequence of pressure adjustments and each sac remains attached to its cell by a very narrow neck. Into the cavity of such a sac is squeezed not only some of the fluid neuroplasm from the nerve cell but also lengths of its neurofibrils which fold and bend upon themselves in such a way as to demonstrate in a most conclusive fashion their complete integrity and independence. It would be difficult to devise a more satisfactory demonstration for neurofibrils as real structures in living nerve cells than this is, for the cells are living and the fibrils are observed under these circumstances and without hardening or staining.

Evidence of this kind points to the correctness of the original observations by Remak and by Schultze and renders it impossible to deny the existence of neurofibrils as constituents of living nerve cells. Incidentally it may be mentioned that although Bozler was the first to call attention in a critical way to the neurofibrils of Rhizostoma, these bodies had already been noticed many years earlier in the living tissues of this jellyfish by Hesse (1895).

Bozler's work is not only important in indicating what neurofibrils are, but it yields very significant results on the general problem of the physical conditions of the materials contained within ganglion cell and nerve fibers. Aside from minute particles such as mitochondria and the like the cytoplasm of nerve fibers and ganglion cells contains in the main two materials, neurofibrillar substance and the intervening neuroplasm or interfibrillar material as it is sometimes called. The older cytologists held a variety of opinions concerning the physical properties of these materials. According to Koelliker (1896), Retzius (1889) and others neurofibrils are filaments of the consistency of a fairly firm gel imbedded in a firm

neuroplasm. Thus the whole axis cylinder of a nerve fiber was believed by this school to be relatively resistant, a condition that was supposed to be characteristic of the substance of the nerve cell also. According to another group of workers represented by such investigators as Leydig (1857) and Nansen (1886), the neuroplasm of cells and fibers was looked upon as a firm material through which the neurofibrils extend as fluid-filled tubules. From this standpoint neurofibrils are not firm bodies but cavities in a matrix of stiff jelly. Finally a third school composed of many investigators including Kupffer (1883), Boveri (1886) and others claimed that neurofibrils are relatively tough filaments in a fluid neuroplasm. It is difficult to harmonize the observations of Bozler with the opinions of any of these groups except the third. The formation and filling of hernia-like sacs and the folding of neurofibrils into such sacs, as clearly shown in Bozler's figures, are conditions hardly to be understood except on the assumption of a fluid neuroplasm and a relatively resistant neurofibril. Such an interpretation of the physical condition of these elements is quite in agreement with the observations of Matsumoto (1920) on the movement of small bodies such as mitochondria in the neuroplasm of growing axis cylinders and with those of Ingvar (1923) on the shifting of the whole neurofibrillar framework through the neuroplasm of centrifuged nerve cells. These and other like observations show beyond a doubt the correctness of the growing conclusion among neurologists that the neuroplasm of nerve cells and of nerve fibers is a fluid in which relatively firm neurofibrils are suspended.

Although the work on living nerve cells compels us to admit that neurofibrils are real constituents of these bodies, it does not oblige us to accept as such all the structural features that have been ascribed to these fibrils by past workers. Most of these features have been described from specially prepared material and as already pointed out there is no certainty that they may not be the

results of the methods of preparation. Before such features can be accepted they must be demonstrated in the living cell.

From what is known of neurofibrils in the living state it may be said that they are relatively firm filaments that form axial systems of parallel members in nerve fibers and complicated intermeshing feltworks in cell bodies. They apparently do not divide nor do they extend beyond the limits of the nerve cell in which they originate. Whether they anastomose and form true networks or not is a question that can not at present be answered. Such unions were not observed by Bozler in Rhizostoma where, had they been present, an excellent opportunity to note them was afforded. At the present moment little more can be said with certainty about the nature of neurofibrils. New discoveries concerning them must await the finding of especially favorable material and the application of novel methods for the study of this material in the living condition.

In passing from the structural aspect of neurofibrils to their functional side we leave a region in which the light, though dim, is certain for one in which almost nothing but obscurity reigns. Most of the neurofibrillists, and this includes such vigorous advocates as Apáthy and Bethe, accepted Max Schultze's dictum that neurofibrils are the transmitting elements of the nervous system. This opinion is almost universal, for only a few workers have expressed other views. Thus von Lenhossék, without, however, denying that neurofibrils may play a part in conduction, regards them as primarily concerned with the mechanics of early development and ascribes to them what might be called a supporting function in the progressive growth of the axis cylinder. That the neurofibrils constitute a purely sustentative system internally bracing the external form of the neurone is the opinion of Koltzoff (1905), Goldschmidt (1910) and von Szüts (1914), who in this respect stand out in strong contrast with most other neurofibrillists.

It is true that neurofibrils are firmer in texture than the neuroplasm that fills much of the space in the nerve cell, but it is also true, as Bozler has pointed out, that this extra firmness is so slight as to be of no real value in forming a skeletal organelle. This conclusion is well supported not only by Bozler's own experimental evidence but also by such observations as those of Ingvar, according to whom the whole fibrillar system of a nerve cell may be broken down by centrifugation without seriously disturbing the form of the cell itself. Thus there seems to be no good reason for assuming that neurofibrils are primarily supporting elements. The neurone maintains its shape chiefly through its outer covering and not in consequence of an inner skeleton.

Having reached this point in his discussion of the function of neurofibrils Bozler ends by stating that since neurofibrils are not sustentative they must by process of exclusion be conductors. This conclusion would be sound if it could be shown that these two functions exhaust the list of possible neurofibrillar activities. But, it may be asked, are there not other possible functions for neurofibrils than mechanical support and nervous conduction?

As intimated at the beginning of this section there is at present almost no real evidence on which to base a conclusion concerning neurofibrillar activities. At best hypotheses may be framed to which future work may or may not give support. To this category belongs the proposition with which this paper closes.

The life of the neurone is a complex one. In consequence of its function of conducting nerve impulses this type of cell has developed enormously attenuated processes which in the form of nerve fibers penetrate the animal body often for great distances. In man, for instance, some of these fibers have a length of a meter or more while their diameters may be only five thousandths of a millimeter or about the thickness of a cobweb. A fiber of approximately such a diameter and one meter in

length is 200,000 times as long as it is thick. This delicate strand is kept alive throughout its whole extent by its connection with the cell body whose nucleus is in some way essential to its continued life, for should it be cut anywhere the part thus separated from the nucleated portion quickly degenerates and dies. This metabolic activity over an enormously attenuated thread is maintained in all nerve cells that they may continue to carry out their very important function of conduction. The motor fibers of the higher animals transmit certainly two and perhaps three sets of independent influences. First of these is the influence that excites activity in a muscle and that is ordinarily spoken of as the nerve impulse. It passes over these fibers at a relatively high rate, exhibits the all-or-none principle, and is the nervous activity that has been studied for a century or more by workers in the field of nerve-and-muscle physiology. There also pass outward over every motor fiber those influences mentioned in this paragraph, that are conducive to growth and repair and that are essential for the continued life of every part of the neurone. These metabolic influences emanate from the nucleated portion of the cell and pass to the uttermost ends of the neuronic processes. Finally motor nerves transmit impulses that excite in muscles not momentary activity but that mild continuous contraction, the so-called state of tonus. What these influences are has been scarcely more than guessed at, but that they are different from the other two types is quite probable. They may, however, depend upon a separate innervation from that concerned with ordinary motivity, in which case we are justified in ascribing to any one class of motor fibers not more than two sets of transmitted influences.

Two of these three possible sets of influences, the ordinary nerve impulses and the metabolic influences, are recognizable in the sensory neurone, where the independence of these influences is clearly shown by the difference in the direction of their conduction. On the distal stretch of an ordinary sensory neurone in the verte-

brates the nerve impulses run toward the central organ, while the metabolic influences run away from it. Hence these two activities must be admitted to be separate.

What parts of the neurone are concerned with these two types of conduction? A final answer to this question can not be given, but a certain amount of evidence is beginning to appear. It is coming to be a recognized fact, as Hanström has recently noted, that the nucleated part of the neurone is its trophic or metabolic center. From this center, as already pointed out, emanate influences that are essential for the continued life of the whole neurone. This nucleated center is what was designated as the ganglion cell by the older neurologists, and to many of them such cells were supposed to be the seat of the central nervous functions. For years this has been a current opinion, but there is as a matter of fact not the least evidence in favor of it. In many instances, such as the bipolar sensory neurones of the lower vertebrates or the bipolar elements in Rhizostoma, as recently described by Bozler, the nucleated part of the neurone lies in the line of nervous transmission. But in a number of other cases, as in the unipolar neurones in higher vertebrates and in some invertebrates, the nucleated portion of the neurone is well to one side of the tract of neuronic nervous transmission. In some of these instances the cell body is attached to this tract by only a slender protoplasmic neck. In this type of neurone the courses taken by the nervous impulses and by the metabolic influences are believed on good grounds to be distinctly different. In the ordinary sensory neurones of vertebrates the nerve impulses originate at the peripheral end, make their way centrally over the neurite, and, without entering the body of the cell, pass on to discharge at the central end of the neurone. The metabolic influences on the other hand originate in the region of the nucleus of the cell body, pass down its neck to the tract of nervous transmission where they separate into two streams, one flowing peripherally over the neurite and the other centrally over the central nerve-fiber process.

This conception of the transmission systems within the neurone is favored by the small amount of experimental evidence that has thus far been brought forward. Bethe (1897) in his study of the nervous system of the crab Carcinus showed that the unipolar cell bodies of a group of neurones in the brain of this animal could be removed without disturbing the reflexes of the second antenna, the muscles of which were innervated by these particular neurones. Hence it may be legitimately concluded, as is implied in the preceding consideration, that the cell bodies of many neurones are not traversed by the nerve impulses but that these impulses take a direct course from the receptive to the discharging end of the element concerned.

If, however, we follow the course of the neurofibrillar tracts within such neurones we find them far from agreeing with those of the nerve impulses. The fibrillar tracts occupy the axes of the neurites but at the junction of the neurite with the unipolar cell process they turn into that process and after passing through it invade the cell body, forming extensive whorls around its nucleus. Or, reversing the description, it may be said that the bundles of neurofibrils start from the region of the neuronic nucleus, pass down the unipolar neck of the cell body and spread from that neck distally and proximally over the fibers of the neurone to their terminations.

Thus the course of the neurofibrils does not follow that of the nerve impulses but does duplicate exactly that of the metabolic influences. I conclude therefore that the neurofibrillar system in the neurone is concerned specifically with the distribution of the metabolic influences and not with the conduction of nerve impulses. These influences start in the region of the neuronic nucleus and spread over the lines of neurofibrils throughout the whole neurone. What the metabolic influences are it is impossible at present to say. It seems hardly reasonable to think of them as streams of material in the nature of a hormone, emanating from the region of the nucleus and

percolating throughout the neurone. They may be chains of ionic readjustment such as have been proposed as an explanation of the nerve impulse. But however we consider them, the hypothesis here put forward assumes that they are what the neurofibrils transmit. This hypothesis further assumes that the neurofibrils are not concerned with the transmission of the nerve impulse, as asserted by Schultze and advocated by Apáthy and Bethe, nor with the mechanical support of the neurone as maintained by Koltzoff and Goldschmidt and in a qualified way by von Lenhossék.

If nerve impulses are not transmitted by neurofibrils what part of the neurone does transmit them? Von Lenhossék (1910) was probably correct in declaring that no specific part of such a cell can be singled out for this special activity. The transmission of nerve impulses, according to him, is rather a function of the neurone as a whole than of any of its parts. And yet certain of these parts are probably more intimately concerned with this operation than others are. The growing belief as to the nature of the nerve impulse is that it is a progressive wave of ionic readjustment in some membranous layer of the neurone (Lillie, 1923). Where this layer is located is not easily determined but that it is about the surface of the neurone is much more probable than that it is buried in its depths. The periphery of the axis cylinder is a much more probable location than the core of that structure. It is well known that if we estimate the metabolism of nerve fibers by the carbon dioxide they give out and designate the amount of this gas discharged by a given quantity of quiescent fiber in a given time as 100 per cent., the increase due to the passage of nerve impulses in the active state of the fiber is only some 15 per cent. (Parker, 1928). These relations are not inconsistent with the belief that the core of the fiber is concerned with maintaining a state of responsiveness on the surface by which the fiber on stimulation is enabled to transmit impulses and to recover from the effects of such

transmission. From this standpoint the core of the axis cylinder is believed to be concerned chiefly with metabolic activities, the periphery with transmission. While this statement is a pure assumption there are no facts so far as I am aware that prevent its acceptance.

The hypothesis that neurofibrils are the parts of the neurone especially concerned with the metabolism of its more distant parts is not only supported by the distribution of these fibrils within the neurone but also by their course of development. It will be remembered that two important facts stand out in the development history of the neurofibrils; first, that their initial appearance is in close proximity to the neuronic nucleus and, second, that they develop while the neuroblast is gradually changing into a neurone and much before nervous functional activity has appeared. Both these facts are without special meaning if we assume neurofibrils to be conductors of nerve impulses, but both are significant if we believe the fibrils to be concerned with metabolic influences, for these influences emanate from the nucleus and must be of first importance in early stages of neuronic growth and before nervous activity has begun.

The idea that the neurofibrillar system of the nerve cell is a part of the metabolic outfit of the neurone is avowedly hypothetical, and yet as a hypothesis it meets at present more of the known facts about the neurone than the hypotheses of nervous conduction or of support appear to do. Hence comes the justification of its presentation, in full understanding that its worth will be determined as our knowledge of the neurone increases.

Postscript. In addition to the fibrillar systems in nerve cells there are two other sets of fibrils that call for some consideration. These are the fibrils in the cells of many ciliated epithelia and in the cells of certain protozoans. In both instances these systems have been interpreted as means of controlling the beat of the cilia or other like parts on the given cells.

The fibrillar system in ciliated epithelia has been recently studied with much care by Grave and Schmitt (1925), who have shown, for instance, that in cells from the foot of the mollusk Lampsilis a fan-shaped system of converging fibrils occurs, the spread ends of which are in contact with the basal bodies of the cilia and the converging ends of which terminate in the deeper part of the cell close to the nucleus. These fibrils, which were long ago described in other similar cells, are believed by Graves and Schmitt to be the means of regulating the metachronous beat of the cilia. But this beat passes over the whole ciliary field without reference to cell limits and it is therefore difficult to understand how such a distinctly cellular mechanism as this could serve as a regulator of such an activity. On the other hand a comparison between the nerve cell with its neurofibrillar system leading from near its nucleus to its peripheral parts and the ciliated cell with a fibrillar system that also starts from close to its nucleus and extends to the basal ends of the cilia is highly suggestive. It is quite possible judging from the connections shown in the ciliated cell that its fibrillar system is in reality a counterpart of that already described for the nerve cell. The nucleus of the ciliated cell is without doubt its metabolic center and the cilia are obviously the parts that would call most heavily on the metabolic resources of the cell. Are not the fibrils, then, that lead from the region of the nucleus to the cilia the most probable parts to be concerned with this transfer of resources? With this view in mind a comparison between them and the neurofibrils of nerve cells seems reasonable.

A second system of fibrils that has recently attracted the interest of investigators is that contained in the cell bodies of certain protozoans. These fibrils have been claimed to constitute what has been called a neuromotor system and the function ascribed to them has been the conductive activities in the control of the cilia, commonly the specialized cilia, of these protozoan cells. From this standpoint they were studied first by Sharp (1914) and

later by Yocom (1918), Kofoid and Swezy (1919), Taylor (1920), McDonald (1922), Rees (1922) and Vissher (1927). All these workers have interpreted the fibrils as parts of a neuromotor system. It has, however, been pointed out that they may serve as a purely supporting mechanism. In Euplotes, Trichomitus and Paramecium the systems concentrate on a center that is located in close proximity to the nucleus, a condition that favors the extension of the hypothesis advanced in this paper for the fibrils in nerve cells and in ciliated cells to those in the protozoan cell. It must not be lost sight of, however, that in two protozoans, Diplodinum and Balantidium, systems of fibrils have been described concentrating on a so-called motorium not located near the nucleus. It is, however, possible that these systems may be something other than those described for Euplotes, Trichomitus and Paramecium in which the nuclear relations are most obvious. It would be difficult to imagine a system better placed for the transfer of metabolic influences from the nucleus to the cilia than that described by Rees for Paramecium and when it is remembered that, as Jennings and Jamieson (1902) long ago showed, fragments of Paramecium continue to exhibit essentially normal ciliary activity for many hours after they have been prepared, it would seem likely that the fibrillar system in this protozoan was more concerned with some such function as that ascribed to it in the present paper than with the immediate control of the ciliary beat.

THE EFFECT OF X-RAYS IN PRODUCING SOMATIC GENOVARIATIONS OF A DEFINITE LOCUS IN DIFFERENT DIRECTIONS IN *DROSOPHILA MELANOGASTER*

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MULLER's work upon the quantitative study of the process of genovariability (= mutability of genes) in Drosophila (Muller and Altenburg, 1919; Muller, 1923 and 1928c) and his recent work on the effect of X-rays in producing genovariations (= mutations), have opened a new field of research in experimental genetics (Muller, 1927, 1928a and 1928b). His chief discovery—the immense acceleration of the process of genovariability through X-ray treatment—has already been confirmed by other authors, partly too on other organisms (Goodspeed and Olson, 1928; Hanson and Heys, 1928; Stadler, 1928; Weinstein, 1928; Whiting, 1928). With the possibility thus presented of a quantitative study of genovariations, there arises a wealth of new problems, as has already been mentioned by Muller. Of particular interest, it seems to me, is the study of reverse genovariations, and in connection with it the possibility of a quantitative study of the genovariability of a definite locus in different directions. The reality of reverse genovariations, of some genes at least, is demonstrated (Demerec, 1928; Timofeëff-Ressovsky, 1925 and 1928). The appearance of reverse genovariations in X-ray experiments is briefly mentioned in the abstract of a paper read by Muller at a meeting of the American Association for the Advancement of Science (Muller, 1928a). Hanson obtained in his X-ray experiments reversions from Bar to full-eye (Hanson, 1928). Gynandromorphs, somatic genovaria-

tions and mosaics show that in many cases the manifestation of some genes can be detected in a part of the body or of the organs which are affected by these genes. The work of Spencer (1926) upon the appearance of pigmented facets in white-eyed Drosophila (as well as different cases of eye-color mosaics) has shown that single eye-facets can be independent of one another in the manifestation of color-factors. If, now, we should use genes which manifest themselves all over the body (*e.g.*, body-colors, bristle- and hair-modifiers, etc.), or genes altering the eye-colors, then, in equal numbers of flies, the probability of detection of a somatic genovariation ought to be higher than that of a germinal genovariation.

With all this in mind I planned experiments which had two purposes: (1) to try to produce by X-raying reverse genovariations of several definite genes; and (2) to try to get quantitative evidence concerning genovariability of a definite locus in different directions. For the first purpose, cultures homozygous for several definite recessive genes were X-rayed. For the second purpose, eggs and larvae of *Drosophila melanogaster* were X-rayed in order to produce somatic genovariations of definite eye-color factors; during the preparations for these experiments the work of Patterson appeared in which the same method was applied for the quantitative study of somatic white-genovariations (Patterson, 1928).

In the present paper the preliminary experiments will be reported, which contain two series: (1) the X-ray treatment of eggs and larvae of homozygous white—and sc w^e ec—flies, in order to produce somatic reverse genovariations of w, w^e and ec; (2) the X-ray treatment of F_1 eggs and larvae from females heterozygous for w; the males obtained from these cultures are of two types: white and red (normal); in examining the eye-facets of the males from these cultures, we can try to compare the genovariabilities of the locus W in two directions: from W to w and from w to W.

The method of treatment was as follows: eggs or young larvae were subjected to X-rays in open, wide

glass cylinders with food. Fifty kv., 5 ma., a target-distance of 17 cm and an aluminum filter 1 mm thick were used. Two lengths of treatment were given to the cultures: 100 minutes (designated as A5) and 120 minutes (designated as A6).

The results obtained are summarized in Tables 1–3. The cultures designated in the tables as "Eggs" are those in which eggs or very young larvae were X-rayed; in the columns marked "Larvae" are summarized those cultures in which larvae of approximately the first two thirds of the larval stage were tested.

TABLE I.—EXPERIMENTS WITH HOMOZYGOUS sc w^e ec CULTURES

Developm. stages rayed and dosages of raying		sc w^e ec flies			Eye-color somatic genovariations	Eye-color somatic reverse genovariations
		♀♀	♂♂	Total		
Eggs	A5	139	112	**251**	–	–
	A6	108	61	**169**	1?	–
Larvae	A5	317	306	**623**	–	–
	A6	191	173	**364**	1?	1?
Total		**755**	**652**	**1,407**	**2?**	**1?**

In Table 1 the experiments with homozygous sc w^e ec flies are summarized. In two cases flies were found in these cultures apparently having some white (unpigmented) facets. One of them was a female from a culture in which the eggs and very young larvae were treated with the A6 dose of X-rays; it had a large area of about fifty facets, which was lighter than the remainder of the eye and contained apparently unpigmented ommatidia. The other fly was a male with about five lighter colored (apparently unpigmented) facets. In both cases the detection and determination of colorless facets was very difficult, because of the very light eye-color of w^e recessives and because of the roughness of the eyes, caused by the gene ec (echinus). One male from "Larvae A6" had three facets which were darker colored than the remainder of the eye; this seems to have been

caused by a somatic reverse genovariation of w^e. Notechinus eye-areas were not found.

TABLE II.—EXPERIMENTS WITH HOMOZYGOUS W-CULTURES

Developm. stages rayed and dosages of raying		w-flies ♀♀	w-flies ♂♂	w-flies Total	Eye-color somatic reverse genovariations
Eggs	A6	109	62	**171**	–
Larvae	A5	415	421	**836**	1
	A6	233	209	**442**	–
Total		**757**	**692**	**1,449**	**1**

In Table 2 similar experiments with homozygous white cultures are summarized. In all 1,449 flies were examined. One male was found in which the right eye had one pigmented ommatidium. A careful examination of the color of this facet showed that it was much lighter than the normal red. This was probably a case of somatic reverse genovariation from w to one of the light red allelomorphs of the W-series.

TABLE III.—EXPERIMENTS WITH F_1 FROM ♀ $\frac{w}{W}$ × ♂ w CROSSES

Developm. stages rayed and dosages of raying		♀♀ +	♀♀ w	♂♂ +	♂♂ w	Total of flies	Eye-color somatic genovariations	Eye-color somatic reverse genovariations
Eggs	A5	201	191	188	209	**789**	1+ (3)	–
	A6	40	52	32	23	**147**	0+ (1)	–
Larvae	A5	330	363	348	327	**1,368**	3+ (7)	–
	A6	215	196	207	176	**794**	2+ (5)	1
Total		**786**	**802**	**775**	**735**	**3,098**	**6+ (16)**	**1**

In Table 3 the experiments are summarized in which F_1 eggs and larvae from the cross, ♀ $\frac{w}{W}$ × ♂ w, were X-rayed. These cultures give two sorts of females: homozygous white and heterozygous for white (phaenotypically red), and two sorts of males: white and red. The eye-facets of all these flies were examined. The 786 heterozygous females showed sixteen white-facet areas. In the cultures in which eggs had been treated, the four

white areas consisted of 16, 28, 33 and 112 ommatidia respectively. Where larvae were treated, the white areas contained from one to nine ommatidia. In one case (from "Larvae A6") a female had five white ommatidia on the right eye and one on the left. The 775 normal (red) males showed six white areas; the one from "Eggs A5" contained twenty-six white ommatidia, and the others from one to three. None of the 802 white-eyed females showed any changes in facet-color. One of the white-eyed males from "Larvae A6" showed three red ommatidia on the right eye.

In summarizing all the above results we have the following. The 1,407 treated sc w^e ec flies showed two somatic genovariations from w^e to w and, apparently, one somatic reverse genovariation from w^e to W. No somatic reverse genovariation of the gene ec appeared. The 2,986 treated white-eyed flies gave one male showing a red area consisting of three red ommatidia. Another white-eyed male showed one pigmented ommatidium which was, apparently, much lighter than the normal red. The 775 normal red males gave six white areas and the 786 heterozygous $\frac{w}{W}$ females gave fifteen females showing sixteen white areas on the eyes.

The conclusions which can be drawn from the above results are as follows:

(1) The occurrence of white areas on the eyes of red-eyed males can be ascribed only to somatic genovariations from W to w; the rate of occurrence of white areas on the eyes in males (6 in 775) is very near to that obtained by Patterson (Patterson, 1928). The rate of occurrence of white areas on the eyes of heterozygous $\frac{w}{W}$ females (which seem, as suggested by Patterson, to be partly caused by chromosomal disturbances and deficiencies) is much lower (16 in 786) than that obtained by Patterson.

(2) The 1,407 treated w^e flies gave only in two cases flies showing white areas on the eyes. The detection of

white ommatidia in w^e eyes is very difficult and uncertain; but nevertheless we can, I think, admit that the rate of somatic genovariation from w^e to w is much lower than that from W to w. It is of great interest to test in this regard other allelomorphs of the W-series.

(3) A somatic reverse genovariation from w to W appeared once in 2,986 treated flies. Once in 1,407 treated flies a reverse genovariation from w^e to W had apparently taken place, and once in 2,986 treated white flies apparently a reverse genovariation from w to a light red allelomorph. It is obvious that the somatic reverse genovariations in the direction from w to W appear very seldom, as compared with the somatic genovariations from W to w.

A large proportion of the flies hatched from treated eggs and larvae were bred further, to look for germinal reverse genovariations. I can only briefly mention some other results of this work, obtained in addition to those above stated. (1) The mortality of eggs and larvae in X-rayed cultures is very high, particularly if eggs are treated. (2) The sterility of flies hatched from treated eggs and larvae (particularly that of females) seems to be lower than that of adult flies treated with corresponding doses of X-rays. (3) Some of the flies hatched from treated eggs, and many flies hatched from treated larvae, showed abnormalities of the abdomen (similar to abnormal abdomen) and wings spread at different angles. Neither of these characters, apparently, is heritable.

Some of the cultures were obtained from Dr. C. Stern, to whom I am indebted for his kindness; the flies were treated with X-rays in the laboratories of the Siemens Concern, and I owe many thanks to Dr. Hausser for his courtesy and to Dr. Stockmayer for help in the X-ray treatments.

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ON THE POSSIBILITY OF INDEFINITE REPRODUCTION IN THE CILIATE DIDINIUM WITHOUT CONJUGATION OR ENDOMIXIS[1]

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CONSPICUOUS among recent advances in protozoology is the gradual extension of the length of survival of infusoria in pure-line cultures. This consistent lengthening of the so-called life cycle is the direct result of a more thorough understanding of the cultural demands of the animals. The evidence at hand indicates that in general the death of infusoria in such cultures is merely the result of the experimenter's inability to supply adequate cultural conditions and not, as formerly held, the result of any kind of senescence inherent in infusorian protoplasm. Indeed, the classic life cycle of the infusoria as propounded by Maupas (88)—a cycle embracing in succession periods of youth, maturity and, unless conjugation intervenes, senescence and death—has been practically annihilated by the demonstrated longevity of a number of infusoria, and it begins to appear that these organisms are capable, when conditions are favorable, of indefinite reproduction without recourse to conjugation.

Of the instances of long-continued cell multiplication in the absence of conjugation, and, at the same time, of the extension of a life cycle through a thorough study of the cultural demands of the organism, that of *Paramecium aurelia* is most notable. Woodruff (26) cultured a race of this ciliate for approximately 11,000 generations in the absence of conjugation, yet no symptoms of lowered vitality in consequence of the prolonged absence of synkaryon formation were observable. The studies of Woodruff and Moore (24) on *Spathidium spathula* illus-

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trate further the importance of an adequate understanding of the environment in pure-line studies, for the cultural cycle of Spathidium was appreciably extended as its special requirements were ascertained, until, finally, over one thousand generations were attained in the absence of both conjugation and endomixis, and the race exhibited no symptoms of depression as a consequence.

The question of vitality in Didinium was considered, though only incidentally, by Mast (17) in the course of his studies on the effects of conjugation and encystment. Mast cultured a race of Didinium for 1,035 generations in the absence of conjugation and encystment. However, he states (p. 347) that "toward the close of the experiment, after a long period without conjugation or encystment, the didinia seemed to lose their accustomed vigor. . . ." Hence, it is not clear from Mast's results whether the loss of vitality was due to environmental factors or to the absence of conjugation, and the following question remained unanswered: Is Didinium capable of indefinite reproduction in the absence of conjugation or endomixis, provided suitable environmental conditions are supplied? This is the question dealt with in the present brief report.

It may be recalled at this point that endomixis in Didinium occurs at encystment (Calkins, 15), and that it has not been observed during the free life of the ciliate, though the writer (28b) examined specimens daily for endomictic changes for a period of over 250 days. Evidently, the prevention of encystment in Didinium precludes the occurrence of endomixis.

In the present study a pure line of *Didinium nasutum* consisting of four sublines was established on September 16, 1926, with progeny of a recently excysted specimen, and was cultured continuously without conjugation or encystment until September 13, 1927—a period of 362 days. The usual pedigree culture technique, comprising daily isolations and transfers to new environments, was

followed throughout the study. The fluid medium employed in the culture of the lines was Hopkins's (28) "modified Ringer solution II" to which well-fed specimens of *Paramecium caudatum* were added as food. The importance of maintaining pure lines of Didinium on a diet of well-fed paramecia has been discussed by the writer in a previous paper (28a). For details of the preparation of the fluid medium used in the present study, reference may be made to Beers, 28b.

For a period of 265 days the present lines were cultured under practically constant environmental conditions, the temperature varying but slightly from 21° C. During this time the question of the occurrence of rhythms was examined critically, and this part of the cultural period has been reported on (28b) from the standpoint of fluctuations in the reproductive rate. After 265 days, culture at a constant temperature was discontinued.

The data in reference to the fission rate—aptly designated by Woodruff (25) as the "most subtle index of metabolism"—are summarized in Table I, in which the average daily fission rates of the four sublines are averaged further for twenty-day periods, with the exception of the last two days. Examination of the third and fourth columns of the table shows that the fission rate was practically constant in the first thirteen twenty-day periods (when the temperature was constant), and that during the last 102 days (when the lines were allowed to take the summer temperature of the laboratory) it was actually higher than in any preceding period. The second column shows that a total average of 1,384 generations per line were produced in the 362 days. From these results, it is clearly evident that the animals under the conditions of the experiment suffered no decrease in vitality as measured by the rate of reproduction.

As regards other physiological and structural conditions which have been shown to be symptomatic of depression in Didinium, such as an increase in the death-

TABLE I

SUMMARY OF RATE OF REPRODUCTION IN A PURE LINE OF DIDINIUM CULTURED IN THE ABSENCE OF CONJUGATION AND ENDOMIXIS UNDER FAVORABLE ENVIRONMENTAL CONDITIONS. NOTE THAT NO LOSS OF VITALITY AS MEASURED BY THE FISSION RATE OCCURRED, EVEN AFTER 1,384 GENERATIONS OF CULTURE

Days of culture	Total average number generations to date	Total average number generations per line for period	Average number generations per line daily for period
20	68.2	68.2	3.41
40	138.6	70.4	3.52
60	210.6	72.0	3.6
80	284.1	73.5	3.67
100	356.4	72.3	3.62
120	428.9	72.5	3.63
140	499.8	70.9	3.55
160	569.5	69.7	3.48
180	642.0	72.5	3.63
200	716.0	74.0	3.7
220	791.6	75.6	3.78
240	866.4	74.8	3.74
260	937.9	71.5	3.57
280	1017.3	79.4	3.97
300	1103.9	86.6	4.33
320	1186.2	82.3	4.12
340	1283.6	97.4	4.87
360	1375.2	91.6	4.58
362	1384.3	9.1	4.55

rate and encystment rate and the production of monstrous and distorted individuals (Beers, 26, 28a), the animals were consistently vigorous and normal. Both the death-rate and the encystment rate were practically zero throughout the experiment, and neither showed any increase as generations passed. Structural abnormalities were wanting entirely.

Thus the outcome of the experiment shows the cultural conditions to have been entirely adequate and favorable. No evidence of depression or degeneration in the absence of conjugation and endomixis was adduced even after 1,384 generations of culture.

It is evident that the single Didinium originally isolated was capable of producing in the absence of conjugation

and endomixis progeny to the 1,384th generation without loss of vitality. Retention of the original vigor of the race to the 1,384th generation suggests potentialities for the production of many more generations and points convincingly to the possibility of indefinite reproduction in Didinium without recourse to conjugation or endomixis, when adequate cultural conditions are supplied. The results indicate, therefore, that neither conjugation nor endomixis is an indispensable feature of the life process in Didinium.

Summary

A pure line of *Didinium nasutum* was cultured under adequate and favorable environmental conditions for 1,384 generations in the absence of both conjugation and endomixis. At the end of this time, when the experiment was discontinued, the animals were fully as vigorous in reference to fission rate, death-rate and encystment rate as at the beginning of culture, and they were quite normal as regards structural details. The evidence indicates that neither conjugation nor endomixis is indispensable for the maintenance of vitality in Didinium, and that Didinium is capable of indefinite reproduction without recourse to either of these processes, provided adequate cultural conditions are at hand.

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HERMAPHRODITISM IN THE COMMON FROG (*RANA TEMPORARIA*)

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The two specimens described in this paper exhibit abnormalities of the genital organs which justify their being termed hermaphrodites. Specimen A has been in the Museum of the Department of Zoology and Comparative Anatomy at University College, Cardiff, for a number of years, but up till now no note has been published of its structure. Specimen B was given to me some years ago by the late Professor W. N. Barker. This second specimen consisted of the urinogenital organs only, which had been dissected out of the animal in which they were found. I am, therefore, not able to give any information as to the secondary sexual characters of this specimen.

Specimen A. This frog exhibits the external secondary sexual characters of an undoubted male, in that it possesses a well-developed nuptial pad on the first digit of each manus. An examination of the urinogenital system shows, however, that it is an hermaphrodite with well-developed oviducts and testes.

The testes are paired and are connected by fully developed vasa efferentia to normal kidneys. The ureters run from the outer edges of the kidneys and bear towards their posterior ends well-developed seminal vesicles. The left testis is on the whole normal in shape, but has an anteriorly projecting lobe of pigmented tissue, the appearance of which suggests that it is ovarian in character.

The right testis is normal in shape but exhibits two deeply pigmented patches, one on the anterior ventral surface and the other on the posterior of the organ.

The cases described by Crew (3, 4) tend to show that these pigment patches represent the end stages of sex

reversal and are, in fact, all that remain of the ovarian tissue.

Well-developed coiled oviducts are present on both sides which open posteriorly to the cloaca. The cloaca, therefore, receives, in an animal which has become predominantly male, the genital ducts of both sexes; that is, the male urinogenital ducts (ureters) and oviducts. The ureters have, therefore, changed their function from that of a urinary duct to that of a urinogenital duct.

There was nothing worthy of note in the other organs of the body.

Specimen B. In this specimen the kidneys, ureters with well-developed seminal vesicles, and the corpora adiposa are normal. The right testis is normal in shape but appears to be rather small, although it is impossible to make any definite statement on this point in the absence of the whole animal. The left testis is normal in shape anteriorly, but is swollen into a large globular structure posteriorly. Vasa efferentia are normally developed on both sides.

The most interesting feature of this specimen is the presence, on the left side only, of a narrow uncoiled oviduct, which could only have been as long as, or very little longer than, the body cavity.

Crew's observations appear to confirm Cole's (2) conclusion that "a good diagnostic feature as regards sex is the seminal vesicle, which has only been observed in hermaphrodites predominantly male." This suggests that the animal from which the organs described here were taken was almost certainly functionally a male.

Discussion

It has been suggested by Huxley (10) and accepted by Crew (4) that the frog is one of those animals in which the sex is determined by the existence of sex-chromosomes. The presence of sex-chromosomes in the frog has not been proved cytologically, but in the Urodele *Necturus* the chromosome constitution is of the female XX,

male XY type. Because of this fact both these authors suggest that cases of hermaphroditism in frogs are in reality cases of sex-reversal and that hermaphrodite frogs are therefore genetically female. Crew states " . . . then these individuals, XX in composition, instead of developing into normal females become transformed into 'somatic' males by the action of some factor or combination of factors which overrides the chromosome constitution."

These theoretical conclusions as to the genetic constitution of frogs appear to be confirmed by the results of breeding experiments carried out by Crew (3) and Witschi (23).

Necturus is a Urodele Amphibian and its genetic constitution might perhaps be considered to hold good for frogs and toads if there were no further evidence on the subject. Stohler (20), however, has worked out the chromosome constitution of three species of toad, namely, *Bufo viridis* Laur., *Bufo calamita* Laur. and *Bufo vulgaris* Laur., and states that in all three species the diploid chromosome number is twenty-two and the haploid number is eleven. This number also holds good for Bidder's organ.[1]

It might well be argued that the toad as an anurous Amphibian is more closely related to the frog than is *Necturus*. It would then follow that, if the frog agreed with *Bufo* instead of *Necturus*, cytologically differentiated androsperms and gynosperms would not be produced.

Further, it is claimed by both Huxley and Crew that the direction of sex-reversal in frogs is from female to male.

In *Triton*, which may be regarded as being more closely related to *Necturus* than is the frog or toad, and pre-

[1] It should be noted that Beccari, C. R., Assoc. Anat. 21, Réun (Liège, 29–31, III, 1926) has claimed twenty-two and twenty-one chromosomes in the male and female, respectively, of *Bufo viridis*. With this Stohler disagrees.

sumably would have the same chromosome constitution as *Necturus,* Champy (1) has found a sex-reversal in the direction of male to female. Champy obtained his result by intensive feeding of his males. As far as I am aware he has not yet bred from a feminized male.

Again, Ponse (17) has found that Bidder's organ, which occurs in both male and female toads, and has been shown by Stohler to have the same chromosome constitution as the testis, is capable of being transformed into ovary when the normal ovary of the animal is removed.

A point which is worthy of note in Crew's paper is that, whereas he ascribes the natural occurrence of hermaphrodites in frogs to the overriding of the sex-chromosomes, he makes no attempt to explain what factor or factors may have served to act as overriders. Surely, if his explanation is correct, it would be reasonable to expect, in any given frog population in which a single hermaphrodite occurs, a good percentage of frogs similarly affected, as presumably they would all have been subjected to the same influences. That this is not the case is shown by the rarity of hermaphrodites amongst frogs.

In the insects sex-chromosomes have been shown to be present in the Hemiptera and Diptera, where the males are heterogamic and the females homogamic; and in the Lepidoptera (families Geometridae and Bombycidae), where the females are heterogametic and the males homogametic. Doncaster (6a) discovered a race of *Abraxas* in which the female chromosome number was fifty-five and the male chromosome number fifty-six.

Yet Goldschmidt (7), working on the gipsy moth, *Lymantria dispar,* showed that, by crossing different races of this moth obtained from different parts of the world, it is possible to produce every stage of intersexuality from a female to a male, or the reverse, in a manner predetermined. It is important to note that these results were obtained with insects in which sex-chromosomes are known to occur. Goldschmidt's conclusion is

that "each sex contains the factors for both sexes and either sex fundament may become active. Which actually does develop depends exclusively on the quantitative relationship of the two factors." He states that the factor for maleness lies in the X chromosome, whilst the female factor, purely maternal, is handed on to every egg *in the cytoplasm* or in the Y chromosome.

Goldschmidt's solution of the problem of sex differentiation may be quoted here:

> Now what is the nature of the reaction which influences differentiation? . . . The only known physiological activity which suits the case is that of the hormones. And hormones secreted by the reproductive organs are actually able . . . to alter the nature of sex differentiation, whilst hormones from the thyroid gland can induce frog tadpoles to metamorphose irrespective of age. We conclude, therefore, that reaction which is conditioned by the sex factors and occurs with definite velocity is the production of hormones of sexual differentiation.

Again the experiments of Meisenheimer (14) on *Lymantria dispar,* in which he transplanted ovaries to castrated males, and *vice versa,* show that the sex organs, and the somatic structures which are characteristic of the sex, can be completely independent of one another. Or, in other words, that the sex, or degree of intersexuality, is fixed at fertilization and can not be altered later by the introduction of the gonad of the opposite sex. Results which agree with those of Meisenheimer have also been obtained by Oudemans,[2] Kellogg,[3] Kopec,[4] and Prell.[5]

Various other factors have been alleged to affect the sex ratio. Amongst these may be mentioned the effects of late fertilization which has been investigated for frogs by Hertwig (8, 9) and Kuschakewitsch (12), and by Pearl

[2] Oudemans, J. Th., "Falter as Rastrierten Raupen," Zool. Jahrb. 12, 1899.

[3] Kellogg, V. L., "Influence of the Primary Reproductive Organs," etc. *J. Exp. Zool., I.* 1904.

[4] Kopeč, V. L., "Untersuchungen über Kastration und Transplantation bei Schmetterlingen." Arch. Entwicklungsmech., 36, 1913.

[5] Prell, H., Über die Beziehungen zwischen primaren und sekundären Sexualcharakteren bei Schmetterlingen." Zool. Jahrb., 35, 1915.

and Parshley (16) for cattle. In considering the results of these investigations it is important to bear in mind the fact that, whether fertilization occurs late or early, the chromosome constitution of the germ-cells would be the same. The most plausible suggestion in these cases would appear to be based on changes in the metabolism of the ovum or spermatozoon, although of course the possibility of a selective death-rate of either gamete must not be neglected.

Witschi (23) by crossing different races of frogs obtained a result closely analogous to that obtained by Goldschmidt for *Lymantria*.

It has been established as the result of a number of workers that in the frog a condition of transitory intersexuality occurs. There are two chief forms of the development of the reproductive organs, which occur in geographically separate races. In one the reproductive organs are male or female from the beginning. In the other the reproductive organs are at first female, but subsequently approximately 50 per cent. become changed into testes (7). In this latter case the 50 per cent. of males should have the chromosome constitution of females.

Again Witschi (23) was able to show that temperature had a very definite effect on the sex ratio of frogs. Some forms at 21° give normal sex ratios in which the sexes differentiate quite early. In another race normal sex ratios were found when the frogs were grown first at a low temperature and later at a higher temperature. The males, however, were transformed from intermediates. At 20° this race produced nothing but females, but at 27° nothing but males were produced through the transformation of the females.

The case of the freemartin in cattle and goats is of interest in any discussion of the sex-chromosomes. Both Lillie (13) and Keller and Tandler (21) have concluded that these intersexes are produced by an anastomosis of the blood-vessels of the foetal membranes, which permits

the male hormone of one twin to enter the female foetus and thus produce the intersexual condition. Keller (11) has also demonstrated the same condition to exist in the case of goat freemartins.

Doncaster (6) has suggested that the tortoiseshell tomcat may be in reality a freemartin, in which masculization is more complete than in cattle, the fertile male being the last stage in a series of intersexes.

Riddle (18) has described a case of complete sex-reversal, from female to male, in an adult ring dove, and Crew (5) earlier described a similar case in the domestic fowl. In both these examples the ovary had been destroyed by tuberculosis. Riddle's opinion on sex determination is "that those conditions or agencies which *increase the metabolism* of gamete or zygote tend to carry development in the male direction," and he suggests "that tuberculosis in particular and the destruction of the ovary in general may effect such an increase in the metabolism."

Crew's conclusion is that "the type of sex-organization and of the reproductive functioning of the individual is not irrevocably decided by the sex-chromosome constitution. It is certain that an XY individual—'a determined female'—can produce sperms just as efficiently as it can produce ova. . . ."

A survey of the foregoing statements, which do not more than touch on the fringe of the question of sex determination, shows that there are extant at least three views on the subject, namely: (a) The sex-chromosome theory, (b) the hormone theory and (c) the metabolic theory. The two latter are perhaps so interdependent that it would be quite feasible to group them together. In any case they remain well marked off from the views of such workers as Morgan (15), who states:

> It is perhaps needless to point out that if, in a species in which sex is determined by chromosome-mechanism, it were possible to change the sex by other agencies in spite of the chromosome arrangement, the latter relation would be entirely thrown out of gear and males would transmit

sex-linked characters and sex itself like females, and females like males. As no such cases have been found *it is futile to discuss such a possibility.*[6]

The overriding of the sex-chromosomes, which Morgan dismisses in a rather summary manner, has now been shown to occur in so many cases as to prompt the question, "Is sex determined by the sex-chromosomes?" The evidence appears to be accumulating in favor of a negative reply. Still the sex-chromosomes have been shown to exist in a very large number of animals and it may in future become necessary to regard them as chromosomes "linked" to the sex (male or female). When this "linkage" is upset then "crossing-over"[7] may take place to the opposite sex.

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THE RELATION BETWEEN LENGTH OF STYLES AND MENDELIAN SEGREGATION IN A MAIZE CROSS

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WHEN maize with sugary endosperm is crossed with certain varieties of pop-corn, commonly known as "Squirrel Tooth" or "Rice," there is always a pronounced deficiency of sugary seeds in the F_2 endosperm generation. This has been repeatedly demonstrated by numerous workers. Approximately 15 per cent. of the F_2 seeds are sugary, while practically all other crosses involving the inheritance of starchy and sugary endosperm yield 25 per cent. recessives in the second generation.

The deficiency of sugary seeds in this cross has been attributed by several writers (2) (4) (5) to differences in rate of growth of pollen tubes bearing the *Su* and *su* gametes, either as a result of these factors *per se* or due to linkage of the *Su* gene with a gametophyte factor which accelerates the rate of pollen tube growth.

Direct evidence that *Su* and *su* pollen tubes exhibit differences in rate of growth is at present impossible to obtain; there is yet no way of distinguishing the two types cytologically. Nor is it possible to determine readily whether the distribution of pollen tubes in the style, with respect to length, is bi-modal, because of the difficulties involved in sectioning or teasing the long styles. If, however, there are pronounced differences in rate of growth of the two classes of pollen tubes, variations in the percentage of recessives might be expected at different levels of the ear. With a constant difference in rate of growth of *Su* and *su* tubes a lower percentage of sugary seeds should be found at the base of the ear as compared to the top, as a result of the greater distance involved.

Such a difference has never been shown, although in the case of abnormal segregation of another endosperm character, attributed by Mangelsdorf and Jones (5) to linkage with the same gametophyte factor involved in Rice Pop, a significant difference was found in the percentage of recessive seeds in the upper and lower halves of the ears. In this case, however, the recessive character was associated, presumably, with the faster growing tubes. An excess, rather than a deficiency, of recessives occurred and this deviation was greater in the lower halves of the ears.

Jones (3) has also shown that in mixed pollination experiments, where a mixture of pollen from Rice Pop and sugary is applied to the silks of both classes, there are marked differences in the proportion of crossed and selfed seeds in the upper and lower levels of the ears.

The present paper represents the results of an attempt to determine (*a*) whether the percentage of sugary seeds in the second generation of Rice Pop $\times$ sugary crosses is affected by the length of the styles, and (*b*) whether the relations between style length and percentages of recessives indicate *constant* or *changing* differences in the rates of pollen tube growth of the two gametic groups.

In the first experiment eighteen F_1 plants of a cross between Rice Pop and sugary, grown at College Station in 1927, were arbitrarily divided into two series. In the first series, hereafter called "normal styled," the silks were cut off three to four inches beyond the tips of the ears. The ears themselves also averaged between three and four inches in length so that the total stylar distance traversed by the pollen tubes was never less than three, never more than eight inches.

In the second series, hereafter termed "short styled," the husks, silks and cob were cut back below the tip of the cob. The husks were partly split open and pollen was sifted down into the mass of silks. Although the longest silks in this series were three inches in length, the stylar

distance involved was probably considerably less, as pollen was scattered over the entire length of the silks and the pollen tubes were given the opportunity to penetrate at any point. In other words the stylar distance in this series was never greater than three inches and the modal distance was probably less than an inch.

Counts of the sugary and starchy seeds of these two series are shown in Tables I and II. The difference be-

TABLE I. SEGREGATION OF STARCHY AND SUGARY SEEDS ON EARS WITH "NORMAL STYLES"

Ear No.	Total seeds	Sugary	Per cent. sugary
4102	193	27	13.99
4103	193	25	12.95
4104	252	38	15.08
4105	121	9	7.44
4106	93	14	15.05
4107	171	8	4.68
4108	82	12	14.63
4109	116	7	6.03
4110	221	37	16.74
4111	237	27	11.35
4112	254	32	12.60
Total	**1933**	**236**	**12.21 ± .50**

TABLE II. SEGREGATION OF STARCHY AND SUGARY SEEDS ON EARS WITH "SHORT STYLES"

Ear No.	Total seeds	Sugary	Per cent. sugary
4113	91	17	18.68
4114	178	40	22.47
4115	222	33	14.86
4116	192	25	13.02
4117	66	6	9.09
4118	211	22	10.43
4119	145	26	17.93
Total	**1105**	**169**	**15.29 ± .73**

tween the two series is $3.08 \pm .88$ per cent. This difference is 3.5 times the probable error and is probably significant, the odds against its chance occurrence being approximately 54:1.

The data from the "short styled" series also indicate that the selective action begins very early. The stylar distance involved in this series is very small, probably only a fraction of an inch, and yet there is a marked deviation from the normal ratio. This suggests that there may be differences between the *Su* and *su* pollen grains in rapidity of germination as well as in rate of growth.

The second study was partly an afterthought. The comparison between the "short styled" and "normal styled" series had indicated rather convincingly that differences in rate of growth did occur. The question now arose whether this difference was a *constant* one, or whether it increased or decreased in proportion to the stylar distance. It was thought that this question might be answered by determining the percentage of sugary seeds at various levels of the ears.

The only material available for the study was a number of ears, grown at New Haven, Connecticut, in 1926, of a cross between Rice Pop and a stock segregating for defective seeds, which was at the same time homozygous for sugary endosperm. The segregation for defective seeds had been determined for a number of the ears and found to be normal, and independent of the sugary character and the gametophyte factor in Rice Pop. The presence of defective seeds, therefore, has no effect on the starchy: sugary ratios. Unfortunately, however, the ears had been pollinated as a matter of routine and nothing is known regarding the stylar distance from the tips of the ears to extremities of the silks or the point at which the pollen tubes entered, nor were the ears all pollinated at the same time, so that environmental differences may also be involved.

The ears were divided into sections of one inch in length by slipping rubber bands over the ear, one inch apart; shelling and classifying the seeds from each section separately. Naturally the last section was not always exactly an inch in length but it was never less than one half or more than one and a half.

The results of this classification are shown in Table III. The ears, unfortunately, were not of the same length. They have been arranged in the table so that the upper one-inch section of each ear coincides.

Averaging the sections separately fails to show any progressive or consistent change in the percentage of sugary seeds. If there is any correlation whatever between distance from the tip and percentage of recessives, it is curvilinear in nature, the trend being first in one direction, later in the other.

Grouping the ears of different lengths separately also fails to reveal any consistent trend, although a comparison of the percentage of sugary seeds in the upper and lower halves of the three groups brings forth the fact that in the six-inch ears the percentage of sugary seeds is higher in the lower portion, while in the four- and five-inch ears the percentage of recessives is greater in the upper halves. The difference, too, is more pronounced in four-inch than in five-inch ears, as shown in Table IV. In no case, however, is the difference significant on the basis of odds calculated by Student's formula.

At first glance it would appear that there is no relation whatever between the percentage of sugary seeds and stylar distance and that these results merely contradict and vitiate the conclusions drawn from a comparison of the "normal" and "short styled" series shown in Tables I and II.

A study of the individual ears indicated, however, that progressive changes from tip to base did frequently occur, but that the trend was sometimes in one direction, sometimes reversed, and in still other cases the percentage of sugary seeds first declined and later advanced.

TABLE III.—SEGREGATION OF STARCHY AND SUGARY SEEDS AT DIFFERENT LEVELS OF EARS FROM A CROSS OF RICE POP × SUGARY

Ear No.	Inches from tip												Totals		Per cent. sugary
	1		2		3		4		5		6				
	Tot.	su.	Tot.	su.	Tot.	su.	Tot.	su.	Tot.	su.	Tot.	su.	Tot.	su.	
3441	105	12	98	12	74	15	84	15	77	10	87	13	**525**	**77**	14.67
3442	58	7	72	11	71	13	75	7	70	11	66	9	**412**	**58**	14.08
3443	47	4	85	9	95	13	96	14	107	17	80	20	**510**	**77**	15.10
3444	43	5	69	13	79	11	74	10	83	12	70	14	**418**	**65**	15.55
3453	37	6	46	6	59	5	62	3	58	7	65	8	**327**	**35**	10.70
3454	29	3	45	5	50	6	47	11	56	3	39	11	**266**	**39**	14.66
3455	30	2	60	10	59	6	64	12	58	7	60	8	**331**	**45**	13.60
3456	21	5	39	5	40	4	49	7	38	4	51	8	**238**	**33**	13.86
3457	32	6	43	5	49	11	46	6	47	12	43	8	**260**	**48**	18.46
3445	86	17	93	9	77	9	86	11	67	8			**409**	**54**	13.20
3446	62	11	85	14	102	16	107	18	89	9			**445**	**68**	15.28
3447	65	6	76	14	73	12	82	7	60	9			**356**	**48**	13.48
3448	65	9	91	11	120	20	116	18	83	10			**475**	**68**	14.32
3449	66	8	63	14	70	13	66	4	65	5			**330**	**44**	13.33
3450	48	7	84	15	103	16	96	17	79	22			**410**	**77**	18.78
3458	29	6	25	6	28	5	28	5	23	3			**133**	**25**	18.80
3459	40	8	48	8	49	8	47	6	22	3			**206**	**33**	16.02
3451	103	17	101	11	137	9	113	13					**454**	**50**	11.01
3460	82	17	69	13	82	13	67	10					**300**	**53**	17.67
3461	54	10	58	11	66	12	44	5					**222**	**38**	17.12
3462	52	5	50	11	40	8	41	8					**183**	**32**	17.49
3463	23	4	26	6	20	3	22	5					**91**	**18**	19.78
Totals	**1177**	**175**	**1426**	**219**	**1543**	**228**	**1512**	**212**	**1082**	**152**	**561**	**99**	**7301**	**1085**	**14.86 ± .28**
Per cent. sugary	14.87 ± .70		15.36 ± .64		14.78 ± .61		14.02 ± .60		14.05 ± .71		17.65 ± 1.09				

TABLE IV. DIFFERENCES IN PERCENTAGE OF SUGARY SEEDS BETWEEN UPPER AND LOWER HALVES OF EARS

Length ears	Total seeds		Sugary seeds		Per cent. sugary		Differences	Odds
	Upper	Lower	Upper	Lower	Upper	Lower	Upper–Lower	Student's
Six-inch ears	1535	1752	210	267	13.68	15.24	−1.56	9.3:1
Five-inch ears	1653	1116	268	155	16.21	13.89	2.32	17.0:1
Four-inch ears	618	632	105	86	16.99	13.61	3.38	5.8:1
Total	**3806**	**3500**	**583**	**508**	**15.32**	**14.51**	**.81**	

These observations suggested at once that the percentage of sugary seeds may at first be progressively reduced as the stylar distance increases, and that this trend after reaching a certain point may reverse its direction, so that later the percentage of recessive seeds increases with the stylar distance. In other words, the pollen tubes bearing the *Su* gametes may proceed at a more rapid rate during the first period of growth, attain their maximum rate, and later be exceeded in rate of growth by tubes bearing the *su* gametes.

This is by no means an unusual growth phenomenon. Early varieties of corn and other plants usually make a more rapid growth in the initial periods of development but are later exceeded in *rate* of growth as well as *total* growth by the later types.

Such a situation, if it occurred in these ears, would naturally be obscured by combining all the ears in one population with their tips coinciding, as no attempt had been made to maintain a uniform distance between the tips of the ears and the extremities of the silks.

If, however, the percentage of sugary seeds first decreases and later increases, it might be expected that a point would be reached where fertilization was accomplished as frequently, or more frequently, by *su* as by *Su* tubes, so that the proportion of sugary seeds would finally attain or exceed the normal 25 per cent.

There are four ears in Table III which bear 25 per cent. or more sugary seeds in any of the one-inch sections. In

three of these ears the high percentage of recessives occurs in the basal one-inch section; in the fourth it occurs in the second from the base. In other words, the high percentage is found in the lower third of the ear in all four ears. This in itself would appear to be significant. If the high percentage of recessives in any section is merely a chance fluctuation, and it might easily be, due to the small number of seeds, then the chances of its occurring in the basal third of any ear are one third. The chances of this same fluctuation occurring in the lower third of all of the four ears are $(1/3)^4$ or 1/81. Odds of eighty to one are not generally declined. As a matter of fact odds against this particular arrangement being purely fortuitous are probably greater than this, as the high percentage of recessives occurred in the lower one sixth of two of the ears and the lower one third and one fifth, respectively, of the remaining pair.

When these four ears are grouped separately with their tips coinciding, it is found that the average percentage of sugary seeds increases progressively from 12.82 at the tip to 24.07 at the base, as shown in Table V.

TABLE V. SEGREGATION AT SUCCESSIVE LEVELS OF EARS SHOWING 25 PER CENT. OR MORE RECESSIVES IN ANY SECTION

Ear No.	Inches from tip											
	1		2		3		4		5		6	
	Tot.	su.	Tot.	su.	Tot.	su.	Tot.	su.	Tot.	su.	Tot.	su.
3443	47	4	85	9	95	13	96	14	107	17	80	20
3454	29	3	45	5	50	6	47	11	56	3	39	11
3457	32	6	43	5	49	11	46	6	47	12	43	8
3450	48	7	84	15	103	16	96	17	79	22		
Totals	**156**	**20**	**257**	**34**	**297**	**46**	**285**	**48**	**289**	**54**	**162**	**39**
Per cent. sugary	12.82		13.23		15.49		16.84		18.68		24.07	

The intervening values, moreover, occur in consecutive order. The chances of these six values, if they are merely chance fluctuations from the mean, falling into a

consecutive order in either direction are 2/720. The odds are three hundred and fifty-nine to one.

The removal of these ears has also made a change in the remaining population. The average percentages of sugary seeds from tip to base are now: 15.18, 15.83, 14.61, 13.37, 12.36 and 15.04.

It should be particularly emphasized that these four ears have been classified separately, not because they exhibit, individually, certain trends, but only because they produced 25 per cent. or more recessive seeds at some point on the ear. If these high percentages are merely chance fluctuations, there is no reason why these ears should show any difference in trend from the remaining population. If they are not chance fluctuations, then we are justified in considering them separately, even though the high percentages are not themselves statistically significant.[1] It should be remembered, too, that 25 per cent. was chosen as the arbitrary point of division on the assumption that the percentage of sugary seeds might at certain points on the ear attain or exceed the normal ratio. There is no reason for believing that these four ears are the *only* ones exhibiting this trend.

There is, of course, no way of knowing the exact region of the pollen tube growth curves represented by any particular ear. Even if the stylar distance were constant it is quite probable that temperature or moisture differences would cause some variation in the rate of growth,

[1] This emphasis would seem to be unnecessary were it not for the fact that objection has been recently made (1) to our procedure in a previous paper (5) of separating segregating progenies on the basis of deviations twice as large as the probable error. This objection reveals a thorough misunderstanding of the laws of probability. If deviations are mere chance fluctuations it matters not whether a difference of three, two, one or a fraction times the probable error is used as a basis of separation. If, however, two groups which are separated on the basis of small differences in one respect later exhibit significant differences in other respects, then the evidence is strong that the original differences were not *all* chance fluctuations, no matter how small or how large these fluctuations may have been in relation to their errors.

so that in combining all the ears into a single group with their tips coinciding, we are undoubtedly obscuring to a large extent any progressive changes in percentage of sugary seeds, associated with stylar distance. Nor does there appear to be any way of sorting the ears into distinct groups except as has been done, accidentally, in combining all those which exhibit a normal or higher percentage of sugary seeds at any one point.

The comparison between "normal" and "short" styled ears has shown that in the initial stages of growth an increase in stylar distance is accompanied by a decrease in percentage of sugary seeds. Table V indicates rather convincingly that there may also be regions where the percentage of recessives increases with the stylar distance. The natural conclusion is that the difference in rate of growth of the *Su* and *su* tubes is not a constant one. Though the *total* growth of the *Su* tubes is greater at practically all points, the *rate* of growth is first higher for the *Su* tubes, later for the *su* tubes. There is also the possibility that the *maximum* growth of which the pollen tubes are capable is greater for the *su* than the *Su* tubes. In other words, if the stylar distance is sufficiently long the percentage of sugary seeds will approach 25 per cent. and may actually exceed it.

It would appear to be a simple test to determine whether this is true by pollinating a series of ears with extremely long silks. This was attempted in 1928, pollinating the silks at a distance of eight inches from the tip of the cob. The results were disappointing, as only one ear produced seed. Though one ear of corn can prove very little, the results are nevertheless interesting. The percentages of sugary seeds borne on each one-inch region from tip to base were 12.0, 12.9, 14.8, 32.0 and 35.7. As the stylar distance to the tip of the ear was eight inches, the one-inch intervals from tip to base of the ear represent pollen tube growth of 9, 10, 11, 12 and 13 inches, respectively.[2] If this ear is typical it would indi-

[2] This is not strictly true because the entire rachis increases in length after pollination and a distance of one inch on the mature ear corresponds to a distance somewhat less than this at the time of pollination.

cate that the percentage of sugary seeds begins to increase gradually after the pollen tubes have traveled nine inches.

It might be supposed that eventually none but *su* pollen tubes accomplish fertilization, so that with stylar distances of about fourteen inches or more the pollinations practically correspond to backcrosses, and 50 per cent. of sugary seeds should be produced. Emerson (2) and Mangelsdorf and Jones (5) have pointed out, however, that the aberrant segregation in this cross is probably not due to the *Su* and *su* genes *per se*, but to a gametophyte factor *Ga* with which the *Su* factor is linked. Evidence that the crossing over between *Ga* and *Su* is of the order of 20 per cent. has also been presented (5). This being true, the percentage of sugary seeds should never exceed 40 per cent. on the average, no matter how great the stylar distance involved. Individual observations, of course, would exceed this limit due to random fluctuations in sampling and to random and inherent variations in the amount of crossing over.

It need scarcely be mentioned that if these results are a true or approximate picture of the change in percentage of recessives with an increase in stylar distance, the procedure of identifying differences in pollen tube growth by determining the percentages of recessives in the upper and lower halves is not a sound one unless the ears represent approximately the same stylar distance. If significant differences between upper and lower halves are shown, a differential rate of pollen tube growth is indicated. On the other hand, a failure to find such differences is not proof that the rate of pollen tube growth is identical. In fact it may be quite possible to have two series of ears, one showing a significant difference in favor of the upper halves, the other a difference in favor of the lower halves, without adopting the fantastic hypothesis that the same gene accelerates pollen tube growth under one set of environmental conditions, retards growth under another (1).

These results, though regarded as preliminary, are published because they suggest a rather surprising situation with regard to the rate of pollen tube growth, and may be of some value to other workers who are studying this problem and similar ones. The writer intends to pursue these studies further, but results may be delayed by the necessity of making up new stocks which are better adapted to Texas conditions than those now available.

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THE SEGREGATION OF ALBESCENT SEEDLINGS AND THE MUTATION TO DEFECTIVE SEEDS IN A PEDIGREE OF THE JAPANESE MORNING GLORY

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MISS YASUI (1920) obtained albino seedlings of the Japanese morning glory, *Pharbitis Nil,* as a simple recessive in its heterozygous pedigree. Later, this form appeared spontaneously in the writer's own culture (Imai, 1924, 1927). In a pedigree of unknown origin, he also observed the segregation of "albescent" seedlings, which appeared to be almost if not quite viable by themselves. The diagnosis of the albescent seedling is as follows: It has cotyledons with a white or whitish smear, which varies in quantity as well as intensity of whiteness, from nearly normal green to almost pure white. The stalks of the cotyledons are generally pale in color, especially on the upper side, which is nearly white. The buds of young leaves appearing between the two cotyledons are distinctly white. The albescent seedling can be detected on inspection from above by observing the color of the bud and stalks of the cotyledons. The white smear on the cotyledons is so variable that it can not always be relied upon for diagnosis. The first evolved leaves of albescent seedlings are usually whitish in color or white-smeared, but those developed later are not diagnostic. The grown-up plants that develop from albescent seedlings are apparently green. The capsules, however, are whitish in color when the seeds are ripening. They are a pale green in color in the normal.

THE SEGREGATION OF ALBESCENT SEEDLINGS

The mother plant, from which these albescent seedlings segregated, developed from a normally green seedling, and had green stems, cordate leaves and white flowers with slightly colored tubes. The progeny were homogeneous in their characteristics, aside from the segregation of the chlorophyll deficient seedlings as shown in Table 1.

TABLE 1

THE DATA OBTAINED IN 1925

The record of the seedling bed

	Green seedling	Albescent seedling	Total	Percentage of albescent seedlings
Observed	112	26	138	18.84

TABLE 2

THE OFFSPRING OF THE NON-ALBESCENTS, RAISED IN 1926

The record of the seedling bed

Family number	Green seedling	Albescent seedling	Total	Percentage of albescent seedlings
11 families	353	—	353	0.00
1	7	3	10	30.00
2	42	9	51	17.65
3	38	6	44	13.64
4	7	3	10	30.00
5	27	10	37	27.03
10	11	2	13	15.38
11	17	2	19	15.26
14	3	1	4	25.00
15	26	8	34	23.53
18	27	5	32	15.63
21	4	4	5	20.00
22	42	10	52	19.23
23	42	8	50	16.00
24	10	2	12	16.67
25	5	1	9	44.44
26	7	1	8	12.50
Total	315	75	390	19.23

TABLE 3

THE DATA OBTAINED IN 1927

Character of mother plant	Family number in the first segregating generation	Family number in the second segregating generation	Green seedling	Albescent seedling	Total	Percentage of albescent seedlings
Green seedling	2	5 families	288	—	288	0.00
		12	89	14	103	13.59
		13	21	5	26	19.23
		14	131	32	163	19.63
		16	45	3	48	6.25
		18	96	21	117	17.95
		20	43	11	54	20.37
		21	60	9	69	13.04
		22	69	17	86	19.77
		23	66	8	74	10.81
		25	126	25	151	16.56
		Total	746	145	891	16.27
	3	4 families	310	—	310	0.00
		5	27	8	35	22.86
		7	72	12	84	14.29
		8	80	15	95	15.79
		9	24	6	30	20.00
		11	15	2	17	11.76
		12	57	8	65	12.31
		Total	275	51	326	15.64
	6	5 families	341	—	341	0.00
	8	8 families	346	—	346	0.00
	22	2 families	48	—	48	0.00
		9	8	1	9	11.11
		10	24	8	32	25.00
		11	21	2	23	8.70
		12	13	3	16	18.75
		Total	66	14	80	17.50
Albescent seedling	2	9 families	—	232	232	100.00
	2	4 families	—	33	33	100.00
	22	8 families	—	169	169	100.00

The proportion of the albescent segregates is only 18.84 per cent. Some of these green and albescent seedlings were transplanted on the field to allow their later growth, but the unfavorable weather damaged all albescents, therefore the data of the next generation are confined to the greens, as shown in Table 2.

The average proportion of the albescent segregates in this generation is 19.23 per cent., which is very close to that of the foregoing generation, though both are conspicuously behind the normal expectation of 3 to 1. The same deficit appeared also in the third segregating generation, the data of which are indicated in Table 3.

In this generation the writer obtained the progenies of albescent segregates and found them constant to type. In the twenty-one families examined, 434 seedlings were recorded, all being albescent. Tables 1 to 3 will show the recessive Mendelian nature of the albescent seedling. The deficiency in the proportion of recessives seems to be constant in each generation. Actually we had:

Year of cultivation	Number of families	Total individuals observed	Average percentage of segregating albescent seedlings
1925	1	138	18.84
1926	16	390	19.23
1927	20	1,297	16.19
Average	37	1,825	17.04

In Table 4 the segregating families through three generations are arranged according to the magnitudes of the deviations in terms of probable errors.

The goodness of fit is high, being $P = 0.781$, and this indicates the fact that the variation exhibited in the proportion of segregating albescent seedlings is due to a chance deviation from an average, 17 per cent. The cause of the deficiency seems to be simple, working either directly or indirectly upon the recessive segregates, which are expected to be produced in a monohybrid recessive ratio. The albescent seedlings are compara-

TABLE 4

A MATHEMATICAL TREATMENT ON THE VARIABILITY EXHIBITED IN THE PROPORTION OF SEGREGATING ALBESCENT SEEDLINGS

Deviations are calculated from the average of 17 per cent.

Dev./Prob. error	−4	−3	−2	−1	0	+1	+2	+3	+4	Total
Observed	0	2	4	12	9	8	1	1		37
Expected	0.79	2.48	5.98	9.25	9.25	5.98	2.48	0.79		37

$\chi^2 = 3.984$ $P = 0.781$

tively less vigorous and they frequently die in their early development after transplantation in the field. The data for the point at issue, as collected in 1927, are indicated in Table 6 and may be summarized as follows:

Type of family	Number of families	Seedlings transplanted on field	Grown-up plants	Percentage of mortality	
				in green seedlings	in albescent seedlings
Normal { true-breeding	5	153	131	14.38	——
Normal { segregating	9	427	359	9.86	45.83
Albescent	7	217	130	——	40.09

The discrepancy in albescent plants seems to be due to their conspicuously weak constitution.

THE APPEARANCE OF ABNORMAL SEEDS

In the second segregating generation of this albescent-segregating pedigree the writer recorded the spontaneous occurrence of abnormal seeds. A number of plants from families Nos. 2, 3, 6, 8 and 22 were transplanted for later growth. The exceptions occurred only in family No. 2. The others gave normal black seeds. At seed-harvest of the plants of this family the abnormal seeds were found in Table 5.

The abnormal seeds are segregated in a simple recessive proportion. The seeds are very thin and deformed

TABLE 5

THE DATA FOR THE MUTATIVE APPEARANCE OF THE DEFECTIVE SEEDS IN FAMILY NO. 2, COLLECTED IN 1926

Family number	Green with normal seed	Albescent with normal seed	Green with defective seed	Albescent with defective seed	Total
2	20	8	7	1	36
	28		8		
Expected	27		9		36

and rusty tan in color. From their form the writer calls them "defective" seeds. The defective seed is a novel character in our morning glory. No description of it occurs in our literature.

The spontaneous segregation of the defective seeds apparently is due to the heterozygosity of the mother plant of this family, presumably being derived through the union of a normal gamete by an abnormal one which had mutated from the normal. The mutation, therefore, occurred from dominant to recessive.

The data for the simple recessiveness of the defective seed obtained in the next generation are given in Table 6.

The data on the segregation of the defective seeds are summarized in Table 7.

The simple recessiveness of the defective seed to the normal is proved to be correct by the data. Table 6 also shows the free assortment of the albescent seedling and defective seed in their simultaneous segregation.

THE DEFECTIVE SEED

Owing to their deformity air-dried, defective seeds weigh only 2.632 grams per 100 grains, on an average, as compared with the normal seeds which attain 4.785 grams. The seedlings from the defective seeds are thin and deformed. They have a higher death-rate, before recovering their vitality after transplantation, as compared with the normals (see Table 6). The grown-up

TABLE 6

THE DATA OF FAMILY NO. 2 FOR THE SEGREGATION OF THE ALBESCENT SEEDLINGS AND DEFECTIVE SEEDS, COLLECTED IN 1927

Character of mother plant	Number of families	Number of plants transplanted into field			Observed number of plants at seed harvest					Percentage of mortality	
		green seedling	albescent seedling	total	green with normal seed	green with defective seed	albescent with normal seed	albescent with defective seed	total	in green seedlings	in albescent seedlings
Green with normal seed	3	91	—	91	64	16	—	—	80	12.09	——
	3	155	32	187	147	—	17	—	164	5.16	46.88
	2	73	23	96	51	13	12	4	80	12.37	30.43
Green with defective seed	2	62	—	62	—	51	—	—	51	17.74	——
	4	127	17	144	—	109	—	6	115	14.17	64.71
Albescent with normal seed	3	—	82	82	—	—	39	—	39	——	52.44
	3	—	123	123	—	—	69	19	88	——	28.46
Albescent with defective seed	1	—	12	12	—	—	—	3	3	——	75.00

TABLE 7

THE SUMMARIZED DATA FOR THE SEGREGATION OF THE DEFECTIVE SEED, IN 1927

Character of mother plant	Number of families	Normal seed	Defective seed	Total
Normal seed	6	203	—	203
	8	196	52	248
Defective seed	7*	—	169	169

plants bear generally more numerous capsules than the normals. They grow vigorously and remain comparatively young later in the season. The deformity of the defective seeds occurs at the drying-up period of their maturation. Prior to this the capsules are filled with swollen seeds. The capsule of the Japanese morning glory is tri-parted, each part containing two seeds. The abortiveness of the ovules is rather commonly found in various varieties of this plant. A statistical survey showed a slight abortiveness of the defective-seeded plants, as indicated in the comparison with the normal in Table 8.

TABLE 8

SHOWING THE VARIATION IN THE SEED NUMBER PER CAPSULE OF THE NORMAL AND DEFECTIVE PLANTS RAISED IN 1927

Number of seeds per capsule	1	2	3	4	5	6	Average
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Normal-seeded	2.53	10.00	16.58	26.08	28.48	16.33	4.17
Defective-seeded	8.24	20.86	26.91	23.93	15.69	4.37	3.31

Their modes are 3 and 5, respectively, indicating the difference in their most common seed number per capsule, and the average seed numbers are 3.31 and 4.17. The low fertility of the defective seed is a zygotic characteristic, and not gametic, as the recessive segregates occur in a proportion approximately regular.

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LARVAL AND POSTLARVAL LOBSTERS

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The matter contained in the short paper now presented has, with the exception of a few necessary references to the results of other scientific investigations, been acquired by personal observation and experimentation over a period of more than ten years. It has been written with a view towards presenting some of the more interesting discoveries which have resulted from the writer's investigations into the habits of young postlarval lobsters, their rate of growth, and various other facts concerning lobster culture in general.

The life-history of the lobster has been studied for many years both in Canada and in the United States as well as in Europe. One of the earliest accounts is that by Professor Sydney I. Smith (1) in the *American Journal of Science and Arts,* vol. 3, for the year 1872.

In this article the author states that there are three stages in the life-history of the American lobster (*Homarus americanus*) which bear little or no resemblance to the adult and one stage which closely resembles the adult. George Ossiam Sars, professor in the University of Christiania, Norway, in 1874, published a paper which contains a figure of the first stage of the lobster. A few years later, in 1895, Professor Herrick, of Adelbert College, U. S. A., published a most exhaustive and beautifully illustrated work on the American lobster.

In the United States experiments in lobster culture have been carried on for many years. In the federal hatcheries at Woods Hole, Massachusetts, and Boothbay Harbor, Maine, no work of this nature is now being done. The former was closed in 1912 and the latter in 1919 after having produced 7,500,000 fry in that year. Likewise in

the state hatcheries of Rhode Island and Connecticut all work relating to lobster culture has been discontinued.

In Canada, on the Atlantic coast, experiments on the hatching of lobster eggs and the rearing of the larvae have been carried on at various places. For a number of years the Canadian Department of Marine and Fisheries operated more than a dozen lobster hatcheries at various points on the Atlantic coast. In these hatcheries eggs nearing maturity, which had been scraped from the female lobster and brought to the hatchery, were placed in jars, through which a steady current of water was kept flowing. As the eggs hatched the larvae rose to the surface and were carried to tanks filled with sea-water. After remaining in these tanks for about three days the lobster fry were taken away and distributed in the ocean. However, the value of these hatcheries became doubtful, and the output as reported was often nothing more than a guess. In 1917 I checked the complete output of one hatchery for the entire season and the output of another hatchery for part of a season. In both, the estimate of the number of fry distributed was obtained by using an automatic counter and actually counting all the fry in one quarter of the tank and multiplying the result by four. The mortality rate was such that out of a total of 27,600,000 eggs less than 100,000 fry were distributed and of the latter only a few would probably reach maturity. As a result of the facts obtained in this work the writer reported that in his judgment the work being carried on in those hatcheries to which his attention had been directed was entirely unsatisfactory, due to the stupendous infant mortality.

Moreover, it is a very noteworthy and significant fact that young lobsters ranging in age from six months to two or three years have been captured on only a very few occasions. Yet it is obvious that such lobsters must exist in very large numbers. As a natural consequence of the difficulty in capturing or rearing lobsters between these ages it has been very difficult for investigators to get re-

liable information on this period of the lobster's existence, and consequently little is definitely known concerning the matter. In the report of the Dominion Lobster Committee of 1898, the chairman, Professor E. E. Prince (2), included a detailed account of the lobster, its development, etc., so far as then known. On page 15 of the latter report attention was drawn to the rarity of the capture of larval lobsters in the sea. In this connection Professor Prince states:

> Considering the countless millions scattered every year through the sea, near the lobster breeding grounds, it is astonishing that so few have been seen or captured. I have myself received specimens of some of the stages described, on three occasions only. They were captured in the Straits of Northumberland, where, during the latter portion of the summer, certain areas must be crowded with various stages. Prior to the capture of my specimens the only actual record in Canadian waters which I can find is that of Mr. J. F. Whiteaves, of the Geological Survey, who eighteen years ago captured specimens half an inch long in the months of July and August off Pictou Island, N. S.

As a rule lobsters extrude the majority of their eggs during the months of July, August and September. For ten or eleven months the eggs are carried on the under side of the abdomen of the female, glued to the swimmerets. They hatch during June, July and August of the following year. During this long period the female guards and protects her eggs, hence she moves about as little as possible and probably does so only in order to procure food. Moreover, during these months she keeps the abdomen and telson folded underneath in order to make a protected place in which to hold the eggs.

The newly extruded eggs are dark green in color and average about 1.53 mm in diameter. This figure was obtained by measuring with a micrometer a number of lobster eggs preserved in formalin. The number of eggs carried by one female lobster varies from ten thousand to eighty thousand eggs, depending upon the size and age of the lobster.

According to several authorities, *e.g.*, Professor Herrick (3) and Professor Mead (4), of the United States,

the following table gives an estimate of the number of eggs.

Length of lobster (Inches)	No. of eggs
8	5,000
10	10,000
12	20,000
14	40,000
16	80,000

After a time the eggs lose their dark green color and change very little in size until the time of hatching approaches. When this time comes they increase in size and show brilliant tints of blue, green and brown. Later on eyes become visible through the deposition of pigment, and the ovum as a whole assumes a transparency so that with a microscope one can see the lobster embryo within the egg case. Hatching of the eggs of a lobster is an operation which takes place over a period of several days. As they hatch the larvae cling at first to the swimmerets of the female, but later release themselves and are dispersed by tides and ocean currents.

In 1917 during the months of July and August, the writer kept twenty berried lobsters in a box specially made for the purpose, the measurements of which were 8 ft. by 10 ft. by 4 ft. The box was placed so that it was about half out of water at low tide. During the six weeks previous to hatching, the eggs were examined regularly, and after hatching once started it continued for a period of approximately ten days. The eggs remained in perfect condition, and a very high percentage successfully hatched. When the eggs are hatched, the young fry, as they are then called, pass through several stages before assuming the adult form. At first they rise to the surface and swim around freely for several days, usually from five to seven days if the water is warm. At the end of this time moulting takes place, the entire outer cover-

ing is shed and the swimmerets make their appearance. The larva continues in this second stage for about five days, during which time it continues to swim at the surface of the water, and then it again moults and passes into the third stage, when the grasping claws are noticeably larger than the walking legs. Up to this time it swims at the surface. In four or five days it again moults and passes into the fourth stage when it assumes the shape of the adult and begins to live at the bottom where it assumes the burrowing habit. During all this time the larva has a ravenous appetite and is decidedly cannibalistic. Other changes occur later, and at the end of one year, after having moulted several times, the lobster is about two and one quarter inches in length.

In 1921 the writer undertook to grow lobster fry in the cold water of Passamaquoddy Bay, at St. Andrews, N. B. The temperature of the surface water during July averaged about 10.5° C., and during August about three degrees higher. For the experiment a box 14 ft. by 10 ft. by 2½ ft. was constructed. It was divided by a partition into two compartments. Screened openings were made in the sides so that there would be a constant circulation of water through the box. Doors were made on the top, and the box was anchored out from the shore and kept floating with one foot of the vertical height out of water. Buoys were attached to the corners. During the day doors were opened to admit light and during the night or when rain was falling they were closed.

Two berried lobsters carrying mature eggs were placed in each compartment, and after the eggs had hatched they were liberated and other berried lobsters substituted. In this way the supply of young fry was kept up. Every day a careful observation was made, both with the naked eye and with the microscope. The eggs hatched quite normally and the young fry remained active at the surface of the water for several days; but at the end of seven days when they should have exuviated for the first time

and passed into the second stage it was observed by examining the larvae with a microscope that this had not taken place, and that many of the fry were infested with both plant and animal growths. The principal diatom found belonged to the genus *Licmophora.* Eighty per cent. of the larvae were practically free from diatoms. The principal parasitic animal preying upon the larvae was a small protozoan—*Acineta mystacina.* These parasitic organisms decreased the vitality of the fry and prevented normal growth.

This experiment was continued for two months, and while some of the fry showed evidences of having passed into the second stage it was impossible to develop them further, and at this point they perished. During the course of the experiment all kinds of available food were tried at various times. At the end of the experiment I reported that in my opinion the chief factor in retarding the growth of the larvae was the coldness of the water. This view corresponded with the fact that during the previous summer I had discovered large numbers of very young lobsters living in water at a temperature of about 18.3° C. The truth of the opinion expressed in 1921 was verified the next year by Dr. Huntsman (5) who successfully reared lobster fry through the successive stages in water at temperatures as high as 25, 20 and 15 degrees C. This goes to show that the temperature of the water has a most important influence upon the growth of lobster fry.

Habits of Young Lobsters

After young lobsters have assumed the adult form they hide at the bottom of the ocean and are very rarely seen until they are at least four or five years old. The writer endeavored to capture young lobsters ranging from one to three years of age, and for this purpose went to Richmond Bay, Prince Edward Island. This body of water is twelve miles long and eight miles wide, thus giving it an estimated area of eighty-four square miles. The

greatest depth as recorded on the admiralty chart is only about fifty feet, but this depth occurs in only a few places. Over a great deal of the area the depth does not exceed fifteen feet and much of the water is under ten feet in depth. Consequently the water is quite warm during the summer months. During July and August, 1919 and 1920, the temperature was as high as 18.3° C. In Richmond Bay, which was found to be an ideal breeding ground for lobsters, I captured young lobsters ranging in size from two and one half inches up to commercial-sized lobsters. During the summer of 1920 two hundred and sixty-three lobsters of very small size were obtained. Most of these were measured, recorded and returned to the ocean, but some were retained for further experiments. Very young lobsters are extremely timid and when captured it is a common thing for them to feign death and remain absolutely rigid and motionless for a considerable length of time.

After walking along the shore for many days when the tide was low and also wading into the shallow water and examining the ocean bottom for signs of lobsters I at last discovered a place where holes appeared in the muddy bottom. After examining many of these I located several small lobsters hiding in these "burrows." When a hole was probed the lobster was forced to leave the burrow. Some burrows had two openings and when the lobster was approached from either opening it made its escape from the other and stirred up the muddy bottom. The water in this place was from two to five feet deep at low tide and the ocean bottom was a mixture of sand and mud with patches of short eel grass. Some days later another colony of young lobsters was discovered but this time the environment was different from that described in the first case. The ocean bottom was rocky and covered partially with seaweed. Lobsters were discovered under the seaweed and also in burrows where the ocean bottom was soft. So far as I know this is the first au-

thentic record of lobsters having been found actually living in burrows.

If Richmond Bay were protected as a breeding ground and no lobster fishing permitted for a number of years I feel sure that as the bay became stocked the large animals would find their way out through the channel into the ocean, and that this migration would continue and greatly benefit the industry at large.

Rate of Growth

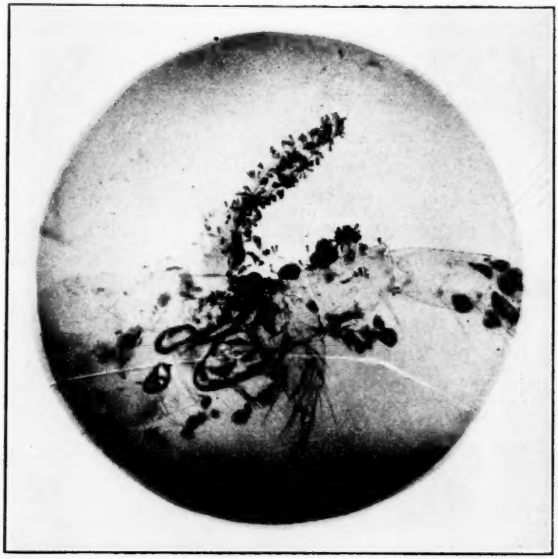
In order to determine the rate of growth of young lobsters a large number was captured as already indicated and the smallest of these were retained for one year. In all 143 were kept. These were divided into three groups, *viz.*, two to three, three to four, and four to five inches in length. A large box was constructed which had the sides and top latticed, leaving quarter inch spaces between the pieces. The measurements were 6 ft. by 6 ft. by 2 ft. This box was divided into three compartments, differing in size because the numbers and sizes of the lobsters kept in them were different. The bottom of the box was covered with stones and sods of short eel grass so as to have conditions as nearly natural as possible and also to have sufficient weight to sink the box. For convenience the compartments were labelled 1, 11, 111.

In compartment 1 seventeen lobsters having an average length of 2.86 inches were placed. In compartment 11 there were sixty lobsters having an average length of 3.60 inches and in compartment 111 there were sixty-six lobsters having an average length of 4.53 inches. The total number retained was therefore 143.

A suitable place was then selected some distance from shore in order to be free from shore ice. The box was properly weighted and so placed that there were several feet of water above the box at low tide.

The problem of feeding the lobsters without bringing the box to the surface was solved by using three collapsible tubes made of strong white duck. One end of

each was nailed around an opening into a compartment, the other ends were fastened together and a weight attached to them to keep the tubes below water. In this way it was an easy matter to haul up the tubes and put food into the box. The lobsters were free to burrow and hide among stones and they could therefore live practically in the same way as if they were free.



Photomicrograph of the leg of a larval lobster covered with diatoms (*Licmophora*) and parasitic animals (*Acineta*).

At the end of one year the compartments were again examined and the following results obtained. In compartment 1, fourteen out of seventeen were found alive and in good condition. The average length of these was 3.97. In compartment 11, thirty-two were found, having an average length of 4.75 inches. In compartment 111, thirty-six were found alive, having an average length of 5.79 inches.

Hence the average length of lobsters in compartment 1 was increased during the twelve months from 2.86

inches to 3.97 inches, an increase of 1.11 inches. In the same way the average length of lobsters in compartment 11 increased from 3.60 inches to 4.75 inches, an increase of 1.15 inches, and in compartment 111 the average length increased from 4.53 to 5.70 inches, an increase of 1.17 inches.

Professor E. W. Barnes (6), superintendent of the Wickford Experimental Station, in a report published in 1910, quotes from a table of the probable rate of growth by Dr. P. B. Hadley (7). In this table the probable lengths are as follows:

Approximate age (years)	Length (inches)
1	$2^1/_8$
2	$4^1/_{16}$
3	$6^3/_8$

Assuming that the three classes retained by me were one, two and three years of age respectively, my result of an increase of 1.11 corresponds with his estimate of 1.12 for yearling lobsters, but during the next two years there is considerable difference.

My results (inches)	Dr. Hadley's (inches)
1.11	1.12
1.15	1.93
1.17	2.31

The work described in this paper was carried on under direction of the Biological Board of Canada.

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(5) *a.* "Natural Lobster Breeding," page 6, 1923.
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(7) See "Methods of Protecting and Propagating the Lobster," by Barnes, pages 94 and 95 for table compiled by Dr. Hadley in 1906.

HIBERNATION OF THE THIRTEEN-LINED GROUND SQUIRREL, *CITELLUS TRIDECEMLINEATUS* (MITCHELL). II. THE GENERAL PROCESS OF WAKING FROM HIBERNATION[1]

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INTRODUCTION

WHILE hibernation literally means to go into winter quarters, it more specifically involves a marked fall in respiration, heart beat and body temperature, resulting in a general immobility. Recovery from this state is slow, compared with waking from sleep. Cold-blooded animals, like frogs and toads, more or less automatically hibernate when the surroundings are cold, since they lack a heat-regulating mechanism. Of the warm-blooded animals none of the birds and only a few of the mammals hibernate. These few are generally among the lower orders of mammals which have apparently failed to develop a perfect heat-regulating mechanism, possibly because survival depended upon the ability of the animal to become cold-blooded during the season when no food is available.

That more information in regard to hibernation is needed is evidenced by the large number of conflicting theories as to its cause, as reviewed by Rasmussen ('16). Misinformation regarding conditions in hibernation and ability of animals to live several years without food may be corrected by presentation of accurate data. Observations reported in the first paper of this series (Johnson, '28) show that in ground squirrels at least metabolism is not entirely suspended and that during one winter of hibernation there may be a 40 per cent. loss in weight.

[1] Contribution No. 103 from the department of zoology, Kansas State Agricultural Experiment Station, Manhattan, Kansas.

The respiration may fall to one in two minutes or even lower, the heart-beat may fall to five a minute in very deep hibernation, while the temperature may fall nearly but not quite as low as that of the surroundings. It seems probable that at the most only a few months could be added to the regular period of hibernation of the ground squirrel without fatal results.

METHODS OF OBSERVATION

Citellus tridecemlineatus pallidus (Allen) from western Kansas was used more extensively than the typical variety, *C. t. tridecemlineatus* (Mitchell). The waking process was studied in torpid animals which had hibernated in the ground in outdoor cages, in caves, in unheated rooms in winter, in refrigeration rooms and in a large refrigerator, which was artificially cooled by means of an automatically controlled Frigidaire installation. Artificial refrigeration made work throughout the year possible and hibernating animals appeared the same under these diverse conditions and seasons. Tendency to hibernate and ability to live in hibernation varied somewhat with the seasons. Whether this variation was caused by an internal seasonal rhythm or by external controllable conditions has not been fully determined.

The methods of observation were similar to those very briefly given in the previous paper (Johnson, '28). Respirations were counted. The beats of the heart above a hundred a minute could be clearly heard with a stethoscope, but the lower rates had to be determined by the use of a fine needle inserted through the thoracic wall into the muscle of the heart. The needle indicated the contractions of the heart by tilting. While the animal was disturbed somewhat by the insertion of the needle and waking was initiated by this, the use of the needle did not apparently modify the rate, except when much probing was necessary to locate the heart or when the animal was only partly torpid. Temperatures were

taken with small specially constructed thermometers and also thermoelectrically by means of thermocouples and a galvanometer. The arrangement of the apparatus is illustrated in Fig. 1. Briefly stated, the galvanometer

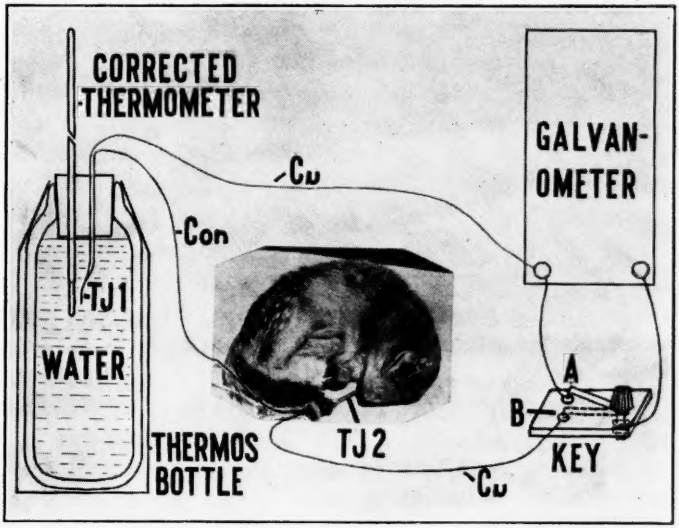

FIG. 1. Apparatus for taking temperatures thermoelectrically with galvanometer and thermocouples. TJ1—thermocouple or thermojunction in the bottle at a known temperature. TJ2—thermojunction in food pouch of the torpid animal which has been removed from its nest and laid on its side. Key—a radio switch making sliding contact. A—short circuit position for zero reading. B—position of key whereby thermojunctions are connected up with galvanometer and deflection of galvanometer produced in proportion to the difference in temperature between TJ1 and TJ2. Cu—copper wire No. 37. Con—Constantin wire No. 36. Wire is insulated and near junction is waterproofed.

deflection is proportional to the difference in temperature of the two thermojunctions (TJ1 and TJ2), the exact relation between deflection and temperature being determined by a number of observations for each pair of thermojunctions used. In most cases 1 mm on the galvanometer scale represented .06 to .08° C. Deflection was thus converted into degrees above or below the

bottle, as the case might be. The error of the apparatus was usually not greater than 0.1° C, and was reduced by having the temperature of the thermos bottle near that of the animal. The temperature of the animal during waking has been taken deep in the rectum at 4 cm, in the anus at 1 to 2 cm, in the esophagus at a point 5 cm from the tip of the nose and in the food pouch. All are valuable, but the pouch temperature was taken chiefly because taking this did not disturb the animal and because it was found to follow the esophageal temperature much more closely than did deep rectal or anal temperatures in waking.

General Description of the Waking Process

The waking process could be induced by handling or warming the animal. Deeply torpid animals with body temperatures of about 2° to 5° C would usually awake in an hour or more if first disturbed and then removed to a warm room of about 22° to 32° C, but might require two, three or even four hours to awake if handled only at first and then left undisturbed in the cold room (0° to 12° C) or if removed undisturbed to a warm room.

The waking activities of the animals were found to fall into two categories dependent upon whether the animal was left rolled up in the nest or was removed from the nest, unrolled and laid on the back or side. No marked difference was found in the waking process of the two varieties used. The accounts given are based not only on general observations, but also upon tabulations of the steps in many such records.

Disturbed awakening outside the nest. When animals were removed from their nests, laid on the side with a thermometer bulb or thermocouple in the cheek pouch (Fig. 1) certain steps in waking in a cold room were noted. The breathing increased in rate, the body showed some movement of forefeet or head or of both, then more marked movements, such as trembling, shivering, shak-

ing the head sideways or jerking it up and down. These movements were accompanied by a gradual straightening of the body from the rolled-up position (Fig. 2, *1*)

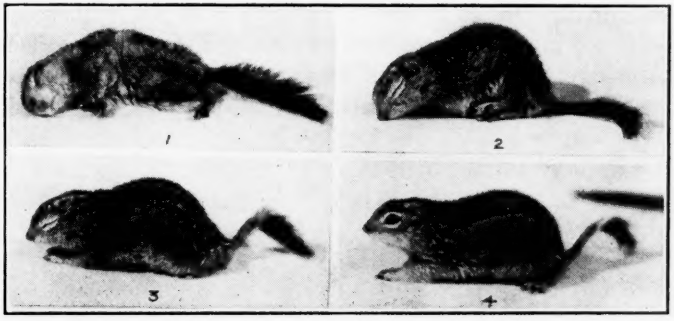

FIG. 2. Stages in waking of a *C. t. pallidus* ground squirrel disturbed by being removed from its nest, laid on its side and taken to a warm room. See description in text.

and were usually followed by deep, rapid and convulsive respirations, not separated by periods of rest as at first. Soon after this the animals raised their heads and tried to get on their forefeet, which usually occurred (Fig. 2, *2*) before they were able to use their hind legs. Before or at the time of getting on all four legs (Fig. 2, *3*) they were able to use their feet in pushing a thermometer out of the food pouch and they would try to bite in a dazed but determined manner when handled, for they were very irritable about that time and at the opening of the eyes, which followed shortly (Fig. 2, *4*). After opening the eyes they could struggle and move forward. The uncertainty and stiffness of the first attempts to walk were gradually lost, but they usually sat quietly for several minutes following waking, after which their movements were normal.

If the animals were removed to a warm room in addition to being laid on the side with a thermometer or thermocouple in the food pouch the steps mentioned were crowded together. The body movements, such as the

getting up on the forefeet, trembling and opening of the eyes, often occurred two at a time and the waking period was shorter.

Waking in the rolled-up position in the nest. The waking process in animals slightly disturbed by having their temperatures taken but which were replaced rolled up in the nests in the cold room, and in animals not disturbed but removed carefully to a warm room was found

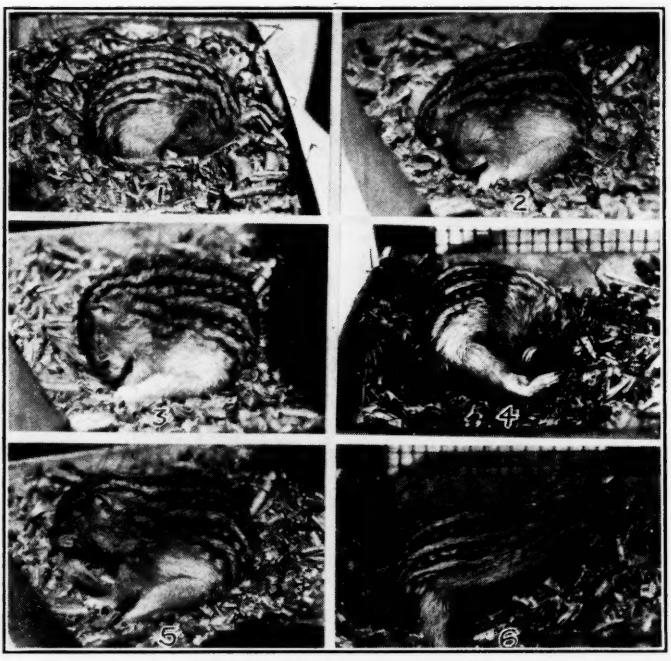

FIG. 3. Typical stages in waking of *C. t. pallidus* animals left undisturbed in nest but removed to warm room to awake. In order to show the animal, the nest materials have been removed from the side nearest the camera. (1) Animal rolled up in nest. (2) Head working out from under. (3 and 4) Different animals showing head out from under but nose down. (5) Head raised. (6) Awake.

to be very much alike. Apparently this was because of the rolled-up condition of the animals in the depressed

nests in the wood shavings in the bottom of the cages (Fig. 3, *1*). There was considerable variability in the progress of waking and some even in the order in which the waking movements appeared. Tabulations of the waking of forty-six torpid *C. t. pallidus* in the warm room were studied. Most of these animals did not go through all the stages already given. The first marked activity of eighteen of these animals was increased respiration, usually moderately deep and rapid, but sixteen animals showed a slight raising or humping of the body without unrolling from the ball-like form and seven others unrolled very slightly, partly working the head out from under as a first activity (Fig. 3, *2*). Both of these motions probably are associated with the need for more oxygen which is difficult to obtain with the nose pressed snugly into the fur of the abdomen. The second stage or activity most commonly noted in the waking of these animals (shown by fifteen of the forty-six) was an unrolling of the body, which involved working the head out from under, the head resting nose down in front against the materials of the nest (Fig. 3, *3*, *4*). The third activity which was most common (shown by eleven animals) was the raising of the head (Fig. 3, *5*) and the most frequent fourth activity was the opening of the eyes (Fig. 3, *6*). Trembling or jerking of the head or body was present in only fifteen animals at some time in the waking process and in nearly all these cases it was slight. The getting up on the forefeet was usually delayed till the opening of the eyes in these animals. Hibernation appeared to pass over into ordinary sleep from which the animal awoke after a time from some stimulus, external or internal.

The rapid disturbed type of waking appears to be that witnessed by most workers, and it was illustrated in *C. t. tridecemlineatus* by Hahn ('14). He gives rapid or convulsive breathing, slow and feeble movements and the opening of the eyes as the chief steps in waking, and states that waking was induced by handling. Dubois

('96) probably had this type in mind when he mentioned the muscular trembling, especially of the cheeks, in the process of awakening of the woodchuck. Dubois also gives the time of awakening as three to four hours, the large size of the animal no doubt preventing the more rapid warming which occurs in the smaller ground squirrel. Shaw ('25a) mentions trembling of the body and shaking of the forefeet in a Townsend ground squirrel removed from its nest and taken to a warm room. Hatt ('27) observed a violent shaking of the head in a ground squirrel, *Callospermophilus,* waking rapidly in the bright sun.

From the waking of undisturbed animals it would appear probable that in nature the awakening in the spring is very gradual so that the animal arouses from a condition of normal sleep and finding the soil conditions favorable digs out to the surface above. Shaw ('25b) considers that the Columbian ground squirrels wake in the spring without assistance from a higher soil temperature than pertains in the winter. Soil temperatures taken in the vicinity of Manhattan, Kansas (McColloch and Hays, '23), show a few decided elevations above 50° F (10° C) at the one-foot depth (where most of the nests of the ground squirrels are likely to be according to Johnson ('17)) in the month of March when the animals come out of hibernation. While other factors such as gonadal activity may stimulate waking, it would seem that for this species and for this vicinity soil temperature is one factor in bringing the animals above ground in the spring.

After waking from hibernation ground squirrels were inclined to fight and frequently some would be found with the occipital region of the skull completely chewed up if several were placed in one cage. This fact and the fighting of animals put in the same outdoor cage in the fall suggest that the animals hibernate in separate dens. Animals which had not hibernated could be put together with only occasional fighting.

During the waking period the temperature, respiration rate and heart-beat rose to normal. The rate of rise in these conditions was observed and graphs made for the purpose of more careful study. This phase of waking will be considered in the third paper of this series.

The Body Temperature at which a Ground Squirrel Awakens from Hibernation and the Time of Waking

The opening of the eyes marked the point at which the animals lost their sleep-like actions and were able to give marked resistance to having the thermometer or thermocouple in the food pouch, and showed definite, if somewhat stupefied, reactions to handling. For these reasons the opening of the eyes may be considered the most satisfactory point at which waking may be placed, for actually the process is gradual. If the animal was waking while

TABLE 1

Temperature and Time (from beginning) of Stages in Waking from Hibernation. Except where Indicated the Animals were Slightly Disturbed by having their Temperatures Taken during the Course of the Observations. All animals Removed from a Cold to a Warm Room (20°–30° C). Ctt—*C. t. tridecemlineatus.* Ctp—*C. t. pallidus.*

Reaction	Variety	No. of animals	Temperature range	Temperature average	Time to reaction in minutes range	Time to reaction in minutes average
Righting	Ctt	12	17–26°	20°	14–87	41
Righting	Ctp	6	13–23°	17°	13–74	47
Opening eyes	Ctt	15	20–32°	25°	17–108	60
Opening eyes	Ctp	12	23–24°	28°	33–99	67
Opening eyes (undisturbed)*	Ctp	45	21–37**	28°	57–264	114
Moving forward	Ctt	9	23–31°	28°	38–109	64

* These animals were undisturbed; their temperatures were not taken until the time they opened their eyes.

** Rectal temperature of twenty-six of these animals taken at the same time averaged 22.3° C and had a range of 15 to 28° C.

lying on its side an earlier point in the process that was more or less definite was the getting up on the forefeet. This may be called the righting reaction. The getting up on all four legs came somewhat later but before the opening of the eyes usually, while the moving forward followed the opening of the eyes.

From Table 1 it is evident that righting, opening of the eyes and moving forward do not occur each at a definite body temperature in different animals. In animals handled slightly one may expect the opening of the eyes to occur usually between the temperatures of 20° and 34° C. The difference between the two varieties in Table 1 may be due to conditions obtaining in the experiment. In the table only the opening of the eyes is given for undisturbed animals because the righting and moving forward reactions often occurred at the same time as the opening of the eyes in these.

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AN ALTERNATIVE METHOD OF DETERMINING CORRELATION COEFFICIENTS FROM CORRELATION SURFACES

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A NUMBER of methods of carrying out the routine of the calculation of the Pearsonian correlation coefficient are now available. Since all give results to any degree of exactness required, the selection of the routine to be followed must be determined by considerations of practicability.

In suggesting a routine which in so far as I am aware is novel, I am not recommending it to replace, but merely to supplement other methods in certain cases. The use of two or more alternative methods has the advantage of providing checks for arithmetical results, and of making clear to students the flexibility of the correlation method.

As has long been known the correlation r_{xy} may be determined without the calculation of product moments when the standard deviations of the sum or difference of x and y are known.

Thus

$$r_{xy} = \frac{\sigma^2 x + \sigma^2 y - \sigma^2 (x - y)}{2\sigma_x \sigma_y} \qquad \text{(i)}$$

$$r_{xy} = \frac{\sigma^2 (x + y) - \sigma^2 x - \sigma^2 y}{2\sigma_x \sigma_y} \qquad \text{(ii)}$$

In most cases in which these formulae are used the standard deviations are determined from independent frequency distributions of x, y, $(x + y)$ and $(x - y)$.

As indicated a number of years ago,[1] the frequency distribution of $(x - y)$ or $(y - x)$ may be obtained from a correlation surface for x and y by the summation of the frequencies in the diagonal cells. This is the essence of

[1] Harris, J. Arthur, "A Short Method of Calculating the Coefficient of Correlation in the Case of Integral Variates." *Biometrika*, 7: 214–218, 1909.

the method suggested twelve years later by Toops[2] and employed in the "Otis Correlation Chart."[3]

Thus summations of the frequencies of x_1y_1, x_2y_2, x_3y_3, . . . , x_py_q, of x_1y_2, x_2y_3, x_3y_4, . . . , $x_{p-1}y_q$, and so forth which interrupt the broken lines of ascending slope on the accompanying diagram give the frequencies of the distribution of $x - y$ or $y - x$. Similarly, summations of the frequencies of x_1y_q, x_2y_{q-1}, x_3y_{q-2}, . . . , x_py_1, and so forth which interrupt the broken lines of descending slope give the frequencies of $x + y$. With these frequencies at hand r_{xy} in terms of x, y and $(x - y)$ is given by (i) and in terms of x, y and $(x + y)$ is given by (ii), or when the table is symmetrical, so that we can replace x and y by x or more specifically by x_1 and x_2, thus denoting that each of the associated variates is used once as a first and once as a second member of the pair, $r_{x_1x_2}$ is given by (iii) and (iv),

$$r_{x_1x_2} = 1 - \frac{\sigma^2_{(x_1 - x_2)}}{2\sigma^2_x} \qquad \text{(iii)}$$

$$r_{x_1x_2} = \frac{\sigma^2_{(x_1 + x_2)}}{2\sigma^2_x} - 1 \qquad \text{(iv)}$$

In case the actual frequency distributions of $(x - y)$, $(x + y)$ are not required, the computations from the correlation surface may be arranged as in the accompanying scheme, in which Σ denotes summation within the arrays.

Let S denote summation of the summations of arrays or of the N individuals of the sample as may be indicated by the context. We may readily deduce in the manner indicated in the accompanying calculation scheme:

$$S(x) = S[\Sigma(x_y)],\ S(x^2) = S[\Sigma(x_y{}^2)] \qquad \text{(v)}$$

$$S(y) = S[\Sigma(y_x)],\ S(y^2) = S[\Sigma(y_x{}^2)] \qquad \text{(vi)}$$

$$S(x+y) = S(x) + S(y) = S[\Sigma(x_y)] + S[\Sigma(y_x)] \qquad \text{(vii)}$$

$$S(x-y) = S(x) - S(y) = S[\Sigma(x_y)] - S[\Sigma(y_x)] \qquad \text{(viii)}$$

$$\begin{aligned} S[(x-y)^2] &= S(x^2) + S(y^2) - 2\,S(xy) \\ &= S[\Sigma(y_x{}^2)] + S(n_x x^2) - 2\,S[x\,\Sigma(y_x)] \\ &= S[\Sigma(x_y{}^2)] + S(n_y y^2) - 2\,S[y\,\Sigma(x_y)] \end{aligned} \qquad \text{(ix)}$$

For $S[(x + y)^2]$ the equations are the same except that the final term is positive.

[2] Toops, H. A., "Eliminating the Pitfalls in Solving Correlation. A Printed Correlation Form." *Jour. Exp. Psychol.*, 4: 234–246, 1921.

[3] Otis, A. S., "Statistical Method in Educational Measurement." New York, 1925.

	$x_1\Sigma(yx_1)$	$x_2\Sigma(yx_2)$	$x_3\Sigma(yx_3)$	. . .	$x_p\Sigma(yx_p)$	
	$n_{x_1}x_1^2$	$n_{x_2}x_2^2$	$n_{x_3}x_3^2$	. . .	$n_{x_p}x_p^2$	
	$\Sigma(y^2x_1)$	$\Sigma(y^2x_2)$	$\Sigma(y^2x_3)$	. . .	$\Sigma(y^2x_p)$	
	$\Sigma(yx_1)$	$\Sigma(yx_2)$	$\Sigma(yx_3)$	. . .	$\Sigma(yx_p)$	
	n_{x_1}	n_{x_2}	n_{x_3}	. . .	n_{x_p}	N
y_9	$n_{x_1y_9}$	$n_{x_2y_9}$	$n_{x_3y_9}$	. . .	$n_{x_py_9}$	n_{y_9}
.	.	.	.		.	.
.	.	.	.		.	.
.	.	.	.		.	.
y_3	$n_{x_1y_3}$	$n_{x_2y_3}$	$n_{x_3y_3}$	. . .	$n_{x_py_3}$	n_{y_3}
y_2	$n_{x_1y_2}$	$n_{x_2y_2}$	$n_{x_3y_2}$	. . .	$n_{x_py_2}$	n_{y_2}
y_1	$n_{x_1y_1}$	$n_{x_2y_1}$	$n_{x_3y_1}$	. . .	$n_{x_py_1}$	n_{y_1}
	x_1	x_2	x_3	. . .	x_p	

$\Sigma(xy_9)$	$\Sigma(x^2y_9)$	$n_{y_9}y_9^2$	$y_9\Sigma(xy_9)$
.	.	.	.
.	.	.	.
.	.	.	.
$\Sigma(xy_3)$	$\Sigma(x^2y_3)$	$n_{y_3}y_3^2$	$y_3\Sigma(xy_3)$
$\Sigma(xy_2)$	$\Sigma(x^2y_2)$	$n_{y_2}y_2^2$	$y_2\Sigma(xy_2)$
$\Sigma(xy_1)$	$\Sigma(x^2y_1)$	$n_{y_1}y_1^2$	$y_1\Sigma(xy_1)$

Formulae involving moments about 0 as origin[4] lead directly to $\bar{x}$, $\bar{y}$ $(\bar{x}-y)$, $(\bar{x}+y)$, σ_x, σ_y, $\sigma_{(x-y)}$, and $\sigma_{(x+y)}$. r_{xy} may be determined by equations (i) and (ii).

While this method of calculating the correlation coefficient is probably better than the antiquated one usually given in text-books, it has no superiority over that of the method of moments about 0 as origin.[5] The latter method is also readily carried out from the accompanying scheme. We note that the value $S[x\Sigma(y_x)] = S[y\Sigma(x_y)]$ leads at once to

$$r_{xy} = \frac{S[x\,\Sigma(y_x)]/N - \bar{x}\bar{y}}{\sigma_x\sigma_y} = \frac{S[y\,\Sigma(x_y)]/N - \bar{x}\bar{y}}{\sigma_x\sigma_y} = \frac{S(xy)/N - \bar{x}\bar{y}}{\sigma_x\sigma_y} \qquad \text{(x)}$$

Further note that the means for the regression lines are given by $\Sigma(x_y)/n_y$, $\Sigma(y_x)/n_x$.

It is also worth noting that Pearson's correlation ratio[6] is readily obtainable in terms of moments about 0 as origin from the present scheme. By definition

$${}_x\eta_y{}^2 = \frac{S[n_x(\bar{y}_x - \bar{y})^2]}{N\sigma_y{}^2}$$

By a series of algebraic conversions,

$$\begin{aligned} S[n_x(\bar{y}_x - \bar{y})^2] &= S[n_x\bar{y}_x{}^2] - 2\bar{y}\,S(n_x\bar{y}_x) + \bar{y}^2\,S(n_x) \\ &= S\left(\frac{[\Sigma(y_x)]^2}{n_x}\right) - 2\bar{y}\,S\,\Sigma(y_x) + N\bar{y}^2 \\ &= S\left(\frac{[\Sigma(y_x)]^2}{n_x}\right) - 2\bar{y}\,S(y) + \frac{[S(y)]^2}{N} \\ &= S\left(\frac{[\Sigma(y_x)]^2}{n_x}\right) - \frac{2\,S(y)\,S(y)}{N} + \frac{[S(y)]^2}{N} \\ &= S\left(\frac{[\Sigma(y_x)]^2}{n_x}\right) - \frac{[S(y)]^2}{N} \end{aligned} \qquad \text{(xi)}$$

[4] Harris, J. Arthur, "The Arithmetic of the Product Moment Method of Calculating the Coefficient of Correlation." AMER. NAT., *44*: 693–699. 1910.

[5] Harris, J. Arthur, *loc. cit.*

[6] Pearson, Karl, "On the General Theory of Skew Correlation and Non-linear Regression." Drapers' Company Research Memoirs. Biometric Series, II. Dulau and Co., London. 1903.

which is essentially the form given without proof by Ayres.[7] This form has a slight advantage over that of the original one suggested by Pearson in that its calculation is free of deviations from means, but it also has the disadvantage of not offering the easy interpretation of its value as does an inspection of the deviations of the means of arrays from the population mean.

Finally the scheme, besides the flexibility which it provides for the calculation of r_{xy}, and the ease with which it provides η and the means for the regression lines, allows for the ready computations of $r_{x(x+y)}$, $r_{y(x+y)}$, $r_{x(x-y)}$, and $r_{y(x-y)}$, which the simpler correlation schemes do not.

The moments from which the constants for x, y, $(x+y)$, $(x-y)$, may be computed have been given above by (v) to (ix). The product moments are derived at once from the scheme

$$S[x(x-y)] = S[\Sigma(x_y^2)] - S[y\Sigma(x_y)] \qquad \text{(xii)}$$
$$S[x(x+y)] = S[\Sigma(x_y^2)] + S[y\Sigma(x_y)] \qquad \text{(xiii)}$$

and similarly for $S[y(x-y)]$, $S[y(x+y)]$.

From this point the calculation is straightforward,

$$r_{x(x+y)} = \frac{\{S[\Sigma(x_y^2)] + S[y\Sigma(x_y)]\}/N - \bar{x}(\bar{x}+\bar{y})}{\sigma_x \sigma_{(x+y)}} \qquad \text{(xiv)}$$

And by similar formulae $r_{x(x-y)}$, $r_{y(x+y)}$, $r_{y(x-y)}$ may be calculated.

The ready calculation of the latter formulae (xii–xiv) is the only material advantage which the scheme has over that offered in 1910, which readily gives the statistical constants in terms of summations of arrays but not in terms of frequencies of sums and differences of the variables.

[7] Ayres, L. P., "The Correlation Ratio," *Jour. Educat. Res.*, 2: 452–456. 1920.

SHORTER ARTICLES AND DISCUSSION

THE QUANTITATIVE THEORY OF SEX

THE opposition hitherto existing between the theories of sex-determination as promulgated by Goldschmidt[1] on the one side and by Bridges on the other would now seem to have become mitigated through the latest investigations by Dobzhansky and Bridges.[2] The views held by Goldschmidt have also thereby gained fresh and strong support and his theories are thus applicable to so widely different organisms as, among others, *Lymantria*,[3] *Rana*,[3] *Gallus*[4] and now finally *Drosophila*.

But, it may be asked, are thereby the questions of sex-determination solved? To find an answer to this question it may be appropriate to recapitulate with a few words Goldschmidt's own results with *Lymantria*. The ultimate sex of every organism is here determined by two factors: (1) the relative dosage of the female- and male-determining substances *F* and *M* (corresponding to *X* and *A* in *Drosophila*); and (2) by the genetic sex of the organism. In Goldschmidt's opinion a genetic female of *Lymantria always* starts its development in a female direction, but according to the relation between *F* and *M* it can from a certain point in its development continue this in a male direction and thus end as a normal female, as an intersexual female or as to a male completely sex-reversed female. In a similar manner a genetic male of *Lymantria* is said *always* to commence its development in a male direction, possibly to revert later in a female direction. Goldschmidt has himself repeatedly emphasized that both these factors cooperate in sex-determination, but in the theoretical discussion the question of the genetic sex appears to have been mainly relegated to the background.

If in studying sex-determination one only wants to look for those forces that determine in which direction the development is to start and not for those forces that may interfere during the progress of the embryonal development, it must be asserted that just in *Lymantria* Goldschmidt's quantitative views play no part. Because, if we call the sex in the direction of which the organism

[1] *Cf.* Goldschmidt and Pariser, *Biol. Zentrbl.*, 43: 446–452, 1923.
[2] Dobzhansky and Bridges, AMER. NAT., 62: 425–434, 1928.
[3] *Cf.* Goldschmidt, *Ergebnisse der Biologie*, 2: 554–684, 1927.
[4] *Cf.* Bonnier, *Arkiv. för Zoologi*, 19B No. 25, p. 1–6, 1927.

begins its development the *primary sex*, then this primary sex in *Lymantria* is entirely determined in the old formal way: *XY* or *XX*. For example, two *Lymantria* organisms may be so imagined that in both the quantity $F = M$. In case of one of them, however, being a genetic female and the other a genetic male, then the primary sex of the former becomes female, and the primary sex of the latter male. The former will develop into an intersexual female and the latter into an intersexual male, that is to say, into two entirely separate forms, although the same relative dosage holds good for both of them: $F\text{-}M = 0$.

In *Rana* conditions are more favorable because it would seem as if facts here could be so interpreted that only *one* primary sex exists, namely, the female. Here all animals possibly commence as females but according to the relative dosage they will continue as females or revert in a male direction. In *Rana*, therefore, it may be imagined that the final sex is *alone* determined by the quantitative values of *F* and *M*. For Goldschmidt's theories of the quantitative values as alone determining the sex to obtain universal application, it is also necessary always to assume the existence of only *one* primary sex. So far as *Lymantria* is concerned, however, no facts seem to point in such a direction and in any case nothing can be said as to which sex in that case would be the primary one. With regard to *Drosophila*, Dobzhansky's and Bridges's investigations go to show that in all intersexes the male sex is the primary one. But should one wish to regard the balance here between the factors determining the male and female sex as the *sole* sex determinator, it would seem unavoidable to conclude also here that normal females too (2N and 3N) commence their development in a male direction but with an early moment of reversion. An investigation into the earlier ontogenesis of the sexual characters in *Drosophila* might possibly clear up this point. Should the outcome of such an investigation go to show that normal females also start as females, the only conclusion to be drawn is apparently this, that the physiology of development follows the principles laid down by Goldschmidt while the chromosome constitution as such determines the primary sex.

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CHROMOSOME COUNTS IN VITIS AND PYRUS

WHILE studying in America on a fellowship of the International Education Board, cytological investigations were carried on in the genera Pyrus and Vitis. The studies were undertaken at Geneva, New York, and Berkeley, California, in 1927 and 1928. The writer is indebted to W. L. Sharp, Cornell University, and T. H. Goodspeed, University of California, Berkeley, for advice and help. As it is necessary to postpone publication of a detailed description of the findings the following list of chromosome counts is given.

LIST OF CHROMOSOME NUMBERS

Name of plant	Diploid chromosome number	Plant obtained from from
Vitis species		
1. Amurense	38	Geneva, N. Y.
2. Coignitiae	38	" " "
3. Doaniana	38	" " "
4. Labrusca	38	" " "
5. Rupestris	38	" " "
Vitis varieties		
6. Bacchus	38	" " "
7. Brighton	38	" " "
8. Catawba	38	" " "
9. Clinton	38	" " "
10. Dunkirk	38	" " "
11. Fredonia	38	" " "
12. Keuka	38	" " "
13. Moore Early	38	" " "
14. Muscat (3 satellites observed)	38	Berkeley, Calif. (Bioletti)
15. Muscat Gigas (6 satellites observed)	76	" " Geneva, N. Y.
16. Niagara	38	" " "
17. Sheridan	38	Berkeley, Calif.
18. Sultanina	38	(Bioletti)
19. " Gigas	76	" "
Pyrus species		
1. Amurense	34	Geneva, N. Y.
2. Baccata	34	" " "
3. Calleryana	34	Talent, Ore. (Reimer)
4. Coronaria	68 ± 2 ?	Geneva, N. Y.
5. Glaucescens	68	" " "
6. Halliana	34	Berkeley, Calif.
7. Ioensis	34	Geneva, N. Y.
8. Niedzwetzkyana	34	" " "
9. Prunifolia	34	Berkeley, Calif.
10. " macrocarpa	34	Geneva, N. Y.
11. Rivularis	34	" " "
12. Sargenti	34	" " "
13. Scheideckeri	34	" " "
14. Soulardi	34	Brookings, S. D. (Hansen)

LIST OF CHROMOSOME NUMBERS—(*Continued*)

Name of Plant	Diploid chromosome number	Plant obtained from from
15. Spectabilis	51 ± 1 ?	Berkeley, Calif.
16. Ussuriensis	34	Talent, Ore. (Reimer)
Pyrus varieties		
17. Cola	70 ± 2 ?	Brookings, S. D. (Hansen)
18. Dolgo	34	Geneva, N. Y.
19. Eden	34	Brookings, S. D. (Hansen)
20. McIntosh	34	Geneva, N. Y.
21. Red Astrachan	34	Davis, Calif.
22. Red Siberian Crab	34	Niles, Calif.
23. " " "	34	Nursery Co. Watsonville (Rodgers)
24. Winesap	34	Niles Cal. Nursery Co.
25. Yellow Newtown	34	Watsonville, Calif. (Rodgers)

In Vitis the counts were made from sections of root tips. The counts in Pyrus were obtained from pollen mother cells and ovary tissue. All sections were stained with gentian violet iodine.

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YELLOW STRIPE—A FACTOR FOR CHLOROPHYLL DEFICIENCY IN MAIZE LOCATED IN THE *Pr pr* CHROMOSOME[1]

IN a culture of six plants of maize grown in 1927, there were noted two plants with characteristic yellow stripes in the leaves. The seed of this culture, grown as a linkage tester, came directly from a culture grown by Professor Emerson in 1926. On examination of the pedigree of the culture in which the yellow stripe plants were noted, it was found that the culture was the F_6 generation of a cross made for the purpose of establishing a certain linkage tester. The probability that yellow stripe was brought in in the original cross and was carried through five generations without appearing is negligible. Assuming that for some reason (such as differential viability) the yellow stripe plants did not appear, the probability of the character being

[1] Paper No. 162, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

brought in by one of the original parents and being carried through the five generations in the heterozygous condition is 0.099. The evidence indicates that yellow stripe behaves as a simple Mendelian recessive with no striking deviations in ratios due to differential viability or other factors. In the four generations previous to the appearance of the character relatively small numbers of plants were grown, but the probability of the plants all being normal merely because of random deviations from expected three to one ratios is slight, the value of D/PE being 6.16 and the probability of the observed deviations having occurred by chance being less than 0.00005. It would therefore seem highly probable that this character arose by mutation from the normal in some one of the selfed generations subsequent to the original cross mentioned above. The factor pair differentiating normal and yellow stripe plants has been given the symbol *Ys ys*.

DESCRIPTION OF YELLOW STRIPE

Yellow stripe is at least in certain respects similar to green stripe described by Emerson (1) and figured by Lindstrom (2). This type of striping appears to bear a close relation to the vascular system of the leaf. Emerson (1) pointed out that in green stripe the tissue of the leaf in close proximity to the larger vascular bundles appears to be of the normal green color while the tissue lying in the regions between the main bundles is lighter green in color. Yellow stripe is of similar nature with the exception that the area between the main bundles is yellowish rather than light green. Yellow stripe varies considerably in the time of its expression. In some cultures it may be noted when the first seedling leaf is well developed. In others it may not become evident until the third or fourth leaf appears. Since yellow stripe becomes evident at an earlier stage when plants are grown in the field than when grown in the greenhouse it was thought possible that ultra-violet light might be a factor in bringing about the expression of the character. Preliminary experiments indicate that exposure to an open carbon arc for short periods has little or no effect on the time of appearance of the characteristic yellow stripes in the leaves. Two soil types were used in these experiments, and it was observed that the character was much more prominent in plants grown in a heavy, fertile soil than in similar plants grown in sand.

Microscopic examination of sections of the living leaf indicates that the plastids of the yellowish areas are normal in number and size but are of a yellowish color. This would suggest a decreased proportion of the green components of the chlorophyll. The constant position of the yellow regions between the larger vascular bundles would suggest a disturbed physiological condition, possibly an inadequate rate of transport of some essential substance to or from the regions between the larger veins.

Linkage Relations

In the F_2 generation of crosses of yellow stripe with linkage testers it was observed that the character segregated as a simple Mendelian recessive. In the F_2 of the cross *purple aleurone, yellow stripe* (*Pr ys*) *by red aleurone, normal* (*pr Ys*), there was apparently a close linkage between *Pr pr* and *Ys ys*. In the culture from purple kernels there were observed twenty-five normal and eighteen yellow stripe plants; and from red kernels, sixteen normal and no yellow stripe plants. No double recessive plants were available for backcrosses, but a yellow stripe plant heterozygous for *Pr pr* was used for crossing on to the normal plants from purple kernels of the F_2 and on the F_1. Counts of plants from these crosses were made in the greenhouse in 1928. All data are presented in Table 1.

TABLE 1

Data showing linkage of *Ys ys* with *Pr pr*

Cross	*Pr*		*pr*	
	Ys	*ys*	*Ys*	*ys*
Pr ys *pr Ys* selfed	98	51	64	0
Pr ys × *pr Ys*	51	73	71	2
	69	73	50	7
	34	63	24	2
	65	114	64	8
Total	**219**	**323**	**209**	**19**
Calc. (8.3 per cent. crossing over)	208.5	369.0	176.5	16.0

The data clearly indicate a relatively close linkage between *Pr pr* and *Ys ys*. The backcross ratios for *Ys ys* show a rather

large deviation from the expected 1 : 1, greater in the case of one culture than in the others. This may be due to the fact that in this lot of material a considerable number of plants were infected with a wilt, and because of their stunted condition plants of the *ys ys* constitution may not have been distinguished from the normal plants. The amount of crossing over between the two loci has been calculated from the *Ys ys* ratio in the red-kernel class. These data give a measure of the ratio of crossover to non-crossover gametes. The percentage of crossing varies considerably, the mean being 8.3. *Ys ys* is being tested with V_2v_2 and V_3v_3, other factors on the *Pr pr* chromosome (3).

SUMMARY

A Mendelian recessive chlorophyll defect in maize is described and named yellow stripe. The factor pair differentiating normal and yellow stripe plants (*Ys ys*) lies in the *Pr pr* chromosome relatively close to the *Pr pr* locus.

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